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Observations on peripheral nerves following local injections of (1) isotonic solutions of varying pH and (2) "iso-pH2" solutions of varying tonicity. *H. M. Weaver*, M.Sc., Ph.D., and *Paul C. Kitchin*, M.S., Ph.D. (Ohio State University) 431

Clinical study of arresting dental caries. *Bert G. Anderson*, D.D.S. (Yale University) 443

Concerning the vitality of calcified dental tissues. II. Permeability of enamel. *William Lefkowitz*, D.D.S., and *Charles F. Bodecker*, D.D.S., F.A.C.D. (Columbia University) 453

Method for determining the specific gravity of dentin and its application to permanent and deciduous teeth. *Julian D. Boyd*, M.D., *Charles L. Drain*, D.D.S. and *Martin L. Deakins*, D.D.S (State University of Iowa) 465

The bacterial flora in different parts of the mouth. *Basil G. Bibby*, B.D.S., Ph.D. (University of Rochester) 471

Studies concerning the oral cavity and saliva. IV. Calcium (1) Calcium content of resting and activated saliva of children. *Robert E. Brawley*, B.S., D.D.S., M.S., and *H. Jobe Sedwick*, D.D.S (University of Rochester) 477

The effect of saliva on lactobacillus acidophilus. *Oliver W. Clough*, D.D.S., M.S., *Basil G. Bibby*, B.D.S., Ph.D., and *George P. Berry*, M.D., Sc.D. (University of Rochester) 493

Destruction of tooth enamel by acidified candies. *Edward S. West*, M.D. and *Frederick R. Judy*, M.D. (University of Oregon) 499

Vascularity of the human enamel organ. *Ellis B. Jump*, D.M.D. (Children's Hospital and Infants Hospital, Boston) 505

Supplement:

I. Sustaining subscribers to the Journal of Dental Research, 1938 519

II. International Association for Dental Research. Register of members by sections, as of Dec. 1, 1938. Compiled by the General Secretary 520

Index 525

General Matter For Volume 17:

Title page i

Contents of the Volume iii

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OBSERVATIONS ON PERIPHERAL NERVES FOLLOWING LOCAL INJECTIONS OF (1) ISOTONIC SOLUTIONS OF VARYING pH AND (2) "ISO-pH" SOLUTIONS OF VARYING TONICITY[1]

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INTRODUCTION

The value of investigative work in the field of materials used in the practise of dentistry is amply demonstrated by the effort on the part of manufacturers to pattern their products along the lines indicated by the results of such research. In recent years considerable work has been done on local anesthetic agents. Much of the investigative work has concerned itself with the speed of anesthetic action as affected by the pH and the osmotic pressure values of the solution. We have, however, been unable to find any reports which are concerned with the morphological reactions of the tissues, in close proximity to the injection, to varying degrees of pH and osmotic pressure values of the injected solution. From the physiological viewpoint it would seem reasonable to require that solutions injected into patients should have pH and osmotic pressure values identical to those of the body tissues. However, it would be more logical if the actual range over which these factors could be tolerated were determined.

The purpose of this investigation is to study the morphologic reaction of peripheral nerve trunks following local injections of solutions of varying pH and osmotic pressure values. It is hoped that this experiment will indicate the tolerance of the tissues to these factors. If, by this investigation, the range of tolerance to pH and osmotic pressure values can be delimited, it would seem plausible, on purely theoretical grounds, that solutions outside these tolerances may be in

[1] Read at the 16th General meeting of the International Association for Dental Research held in Minneapolis in March, 1938.

whole or in part responsible for the after-pain which is of such frequent occurrence. For purposes of discussion the paper can best be divided into two parts.

THE EFFECT OF pH

MATERIALS AND METHODS

A series of phosphate buffers[2] made up in 0.9 per cent NaCl solution covering the desired pH range was prepared and the freezing point depressions of a number of them determined by the method of Johlin ('31). Assuming that mammalian blood is isotonic with 0.9 per cent NaCl solution, one can calculate the tonicity of the buffer solutions; for over the narrow concentration range involved, the tonicity is directly proportional to the freezing point depression. The freezing point depressions of the buffer solutions indicated

TABLE I

SOLUTION	CONSTITUTION	pH	HEMOLYSING ACTIVITY (PER CENT)
1	Phosphate Buffer Solution	4.37	39.9
2	" " "	5.82	00.0
3	" " "	6.43	00.0
4	" " "	6.80	00.0
5	" " "	7.18	00.0
6	" " "	8.77	00.0

that they were all hypotonic. The tonicity of these hypotonic solutions was raised to unity by the addition of hypertonic NaCl solution of such concentration (3.5 per cent) that 2 cc. were required to raise the tonicity of 10 cc. of the buffer. It was to be expected that the addition of this 3.5 per cent NaCl solution would change slightly the pH of the buffer solutions. Therefore, this character was redetermined in the adjusted buffer solutions, using a glass electrode apparatus.

It is realized that the prepared buffer solutions were not exactly isotonic with 0.9 per cent NaCl but, since they were injected without dilution, it could be predicted confidently that any resulting hemolysis would not be due to osmotic pressure factors but would be a hydrogen ion concentration effect. The tonicity of these solutions with the blood of the experimental animal was tested in the following manner: Blood freshly drawn from the cat heart was washed 6 times with 0.9 per cent NaCl solution. The cells were centrifuged after each washing. Following the final washing the cells were diluted with 0.9 per cent NaCl solution to the original volume of the blood sample.

Thirty drops of the suspended cells were added to 60 cc. of distilled water in order to prepare the 100 per cent standard. Dilutions of this standard were made with the requisite amounts of distilled water to get 75 per cent, 50 per cent, and 25 per cent hemolysis

[2] The writers are indebted to Dr. Ellis J. Robinson, formerly of the Department of Physiology, College of Medicine, The Ohio State University, for his advice and help in the preparation of the solutions used in this investigation.

color. Four cc. of each of the buffer solutions were placed in individual Kahn tubes and 2 drops of the blood cell suspension were placed in each tube. The tubes were then placed in cracked ice for 20 minutes, following which they were centrifuged for 5 minutes,

TABLE II

LEG	INJECTION FLUID			
	Solution No.	Constitution	pH	Hemolysing Activity (per cent)
		Animals 40–49 Normal Controls		
Right	None	———	—	———
Left	None	———	—	———
		Animals 1–10 Experimental Controls		
Left	7	0.900 per cent NaCl Solution	6.98	00.0
		Animals 60–69 Experimental Controls		
Right	8	0.900 per cent NaCl Solution	6.07	00.0
		Animals 11–20		
Right*	1	Phosphate Buffer Solution	4.37	39.9
Left	6	Phosphate Buffer Solution	8.77	00.0
		Animals 21–29		
Right	2	Phosphate Buffer Solution	5.82	00.0
Left	5	Phosphate Buffer Solution	7.18	00.0
		Animals 31–39		
Right	4	Phosphate Buffer Solution	6.80	00.0
Left	3	Phosphate Buffer Solution	6.43	00.0

* Injections caused much pain and were accompanied by numerous muscle twitches.

at 2700 R.P.M. Hemolysis was judged by comparing in a colorimeter the supernatant fluid from the Kahn tubes with the nearest color standard described above.

Table I illustrates the essential features of the buffer solutions used.

For this investigation healthy, young adult cats of both sexes were chosen as the experimental animal. The present experiment together with work to be reported in the future,

necessitated animals whose nutritional background was such as to tend to eliminate nutritional deficiency diseases, and which also possessed a region where the cytological and histological features would be similar to the pterygomandibular triangle in the human. The cat was the animal which fulfilled nearest the above requirements.

EXPERIMENTAL PROCEDURE

The following injection procedure was routine for each animal. The hair about the injection point was cut close with scissors, the skin swabbed with tincture of iodine and then wasned with 70 per cent alcohol. Sterile glass syringes and needles were filled with the various solutions to be injected and placed in an oven at 38°C. long enough to insure the temperature of the fluid being the same as the oven. All solutions were sterilized in an autoclave. Two cc. of fluid was injected slowly into the upper aspect of the popliteal fossa. In each case the attempt was made to pass the needle between the biceps femoris muscle medially and the semitendinous muscle laterally—the needle to penetrate as far as the point at which the sciatic nerve divides into its tibial and common peroneal branches.

The animals were cancelled after 27 hours by an intraperitoneal injection of 10 cc. of standard veterinary nembutal. Immediately after death an incision was made over the injected area and a small segment of the sciatic nerve was removed and placed in 10 per cent neutral formalin. At the same time a small piece of the areolar connective tissue adjacent to the nerve was removed and placed in Zenker's solution. Observations on the connective tissue will be reported at a later date.

After fixation in 10 per cent neutral formalin for 24 hours or longer the nerve trunk was frozen, sectioned longitudinally, mounted in glycerin and studied under polarized light. The polarized light method is fully described in the papers of Setterfield and Sutton ('35), Setterfield and Baird ('36), and Setterfield and Weaver ('37). The normal nerve fiber is characterized by certain regions which are birefringent in polarized light, whereas the remaining structures in the fiber are isotropic under the same conditions. As degeneration of the nerve fiber progresses the nerve loses its birefringency in direct proportion to the degree of degenerative change.

Table II summarizes the experimental procedure of this phase of the investigation.

MICROSCOPIC OBSERVATIONS

Microscopic examination in polarized light of the nerve trunks of the animals revealed evidence of degenerative change in the following cases: the left nerves of animals 6, 22, 36, and 37, and in the right nerves of 15, 20, 38, 44, 60, 68 and 69. In every case the degenerative changes observed were slight in both quantity and quality, and appear to be reversible in type (*fig. 3*). The alteration from the normal (*figs. 1 and 2*) was confined to swelling, vacuolization and peripheral irregularity of the non-neurofibrillated portion of the axis-cylinder.

In attempting to ascertain the significance of the observations reported, several possible causes for nerve fiber degeneration must be considered. It is well known that many systemic diseases of bacterial and virus etiology as well as nutritional deficiency diseases are ac-

companied by degeneration of peripheral nerve fibers. In these cases one would be justified in anticipating degeneration of a bilateral character. None of the animals in the series just reported showed degenerative changes in both sciatic nerves. This observation favors the elimination of these factors as possible causes of the pathosis in

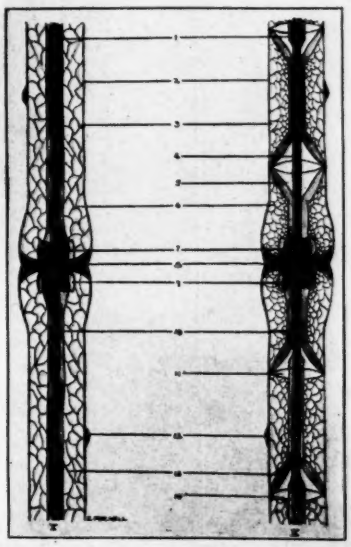

FIG. 1.[3] Semi-diagrammatic drawings illustrating range of normality of normal myelinated nerve fibers as seen by polarized light method. White areas indicate birefringence whereas black and gray regions are isotropic. 1. Non-neurofibrillated portion of the axis-cylinder. 2. Sheath of Schwann. 3. True myelin. 4. Naked true axis-cylinder. 5. Infundibulum. 6. Neurokeratin trabecula. 7. Swollen part of non-neurofibrillated portion of axis-cylinder. 8. Cementing disk. 9. Bracelet of Nageotte. 10. True axis-cylinder. 11. Ostium of infundibulum. 12. Nucleus of Schwann cell. 13. Incisure of Schmidt-Lantermann. 14. Infundibular ring.

question. The pressure resulting from the injected solutions must be considered. As a control for this possibility 20 animals were injected with sterile 0.9 per cent NaCl solution in the same volume and under circumstances identical to the injections of the experimental solutions. Any tissue alterations following these injections should not be caused

[3] Taken from H. E. Setterfield and H. M. Weaver, *Ohio J. of Science*, **37:** 65, 1937.

FIG. 2. Longitudinal section of sciatic nerve of cat 40 (R). Photographed in polarized light.

FIG. 3. Longitudinal section of sciatic nerve of cat 69 (R). Photographed in polarized light. Light degree of degenerative change shown in this section is illustrative of all pathologic change noted in this paper with exception of that described in right nerves of 50–59 series.

by the chemical or physical properties of the solution but rather by the pressure of injection. Of the 20 animals so subjected 4 (20 per cent) showed degenerative changes identical to those described above. The appearance of the pathological changes in these fibers is not indicative of severe traumatic injury, and would simulate only the earliest changes characteristic of typical Wallerian degeneration. It should be pointed out, however, that the writers are unaware of any

FIG. 4. Longitudinal section of sciatic nerve of cat 51 (R). Photographed in polarized light. This photomicrograph illustrates extent of pathologic change described in 50 (R)–59 (R) series.

description, based on a polarized light study, of peripheral nerve fibers following non-perforating trauma of the nerve trunk. Direct trauma of the nerve due to perforation by the needle must be considered. This is more or less highly improbable when one considers the difficulty encountered in making injections directly into a nerve which is not firmly anchored.

In the nerve numbered "38 right leg" the degenerative changes indicated can possibly be explained by circulatory disturbance in the nerve resulting from a large hematoma found at the site of injection.

The observations noted above, together with the inconstancy of the results obtained in any one series of animals, lead the writers to conclude that the degenerative changes in the nerve fibers were not primarily due to hydrogen ion concentration effects.

THE EFFECT OF OSMOTIC PRESSURE

It should be pointed out that theoretically the osmotic pressure of the fluid environment of the nerve trunk may affect this latter structure in at least 2 ways; 1) by a direct effect on the trunk itself, or 2) by altering the circulation and composition of the blood and lymph in its immediate vicinity and thereby, producing nutritive-deficiency, oxygen-lack and metabolic-poison changes.

MATERIALS AND METHODS

The solutions used in this portion of the experiment were prepared by combining varying percentages of sterile 0.9 per cent NaCl solution with sterile distilled water. Table III lists their essential features.

A consideration of osmotic pressure, as a factor in the cause of tissue irritation, is somewhat complicated by some differences in the pH of the different numbers of the series. The range of pH of these solutions extends from 5.98 to 6.98. From the results of the preceding work dealing with pH, however, we are inclined to believe that the buffer action of the tissues would tend to minimize this as a factor in the etiology of irritation.

EXPERIMENTAL PROCEDURE

The experimental procedure of this phase of the problem differed from that described under "Effects of pH" only in the nature of the solutions injected. Table IV summarizes the data involved here.

MICROSCOPIC OBSERVATIONS

Microscopic examination by the polarized light method on the nerve trunks of the experimental animals revealed evidence of degenerative change in the following cases: the left nerves of animals 6, 50, 52, 54 and 60, and in the right nerves of 44, 50, 51, 54, 55, 56, 57, 59, 60, 68 and 69.

With the exception of the right nerves of the animals in the 50 series, the picture of degeneration presented was identical with that described for the pH series. In the right nerves of the animals of the 50 series, the degenerative changes were somewhat more marked and of greater extent, though in no case did the appearance simulate that seen in typical traumatic injury, even of a rather mild character, and in no case did the lesion involve the true axis-cylinder or the myelin sheath (*fig. 4*).

In this series three cases, i.e., animals 50, 54 and 60 showed degenerative changes that were practically identical in both sciatic nerves. This makes it probable that the degenerative changes in these cases were due to some systemic alteration unknown to us.

Experimental controls were run on the possible effects of pressure resulting from the injected solution. Twenty animals were injected with sterile 0.9 per cent NaCl solution in the same volume and under circumstances identical to the injections of the experimental solutions. Of the animals so subjected 4 (20 per cent) showed degenerative changes similar to those described under *Effect of pH*. One of the animals showed similar degenerative changes in both sciatic nerves. In that case the change might be explained by some systemic alteration. If we disregard the bilateral case, the etiology of

TABLE III

SOLUTION NO.	CONSTITUTION	pH	HEMOLYSING ACTIVITY (PER CENT)
7	0.900 per cent NaCl Solution	6.98	00.0
8	0.900 per cent NaCl Solution	6.07	00.0
9	0.675 per cent NaCl Solution	5.98	24.6
10	0.450 per cent NaCl Solution	6.25	64.0
11	0.225 per cent NaCl Solution	6.69	70.0
12	Distilled Water	6.95	100.0

15 per cent of the degenerative changes noted in this part of the experiment could be referable to pressure resulting from the injection.

Of the nerves showing degenerative changes we believe that all can be disregarded, excepting the right nerves of the number 50 series, because the percentage of nerves showing changes in any one series of animals is less than, or only equal to, the percentage of degenerative change noted in the experimental controls.

By referring to Table IV it can be seen that the right legs of animals 50–59 were injected with a solution of 0.675 per cent NaCl solution having a pH of 5.98 and a hemolysing activity, *due to salt concentration*, of 24.6 per cent. Of this group 7 (or 70 per cent) showed degenerative changes which were more clearly marked than any noted elsewhere in this experiment. As to the possible etiology of the change: It has been noted already that 2 members of this series

showed degenerative changes in both sciatic nerves which could be the result of some unknown systemic alteration. According to our

TABLE IV

LEG	INJECTION FLUID			
	Solution No.	Constitution	pH	Hemolysing Activity (per cent)
		Animals 40–49 Normal Controls		
Right	None			
Left	None		—	—
		Animals 1–10 Experimental Controls		
Left	7	0.900 per cent NaCl Solution	6.98	00.0
		Animals 60–69 Experimental Controls		
Right	8	0.900 per cent NaCl Solution	6.07	00.0
		Animals 50–59		
Right	9	0.675 per cent NaCl Solution	5.98	24.6
Left*	11	0.225 per cent NaCl Solution	6.69	70.0
		Animals 60–69		
Left†	10	0.450 per cent NaCl Solution	6.25	64.0
		Animals 1–10		
Right‡	12	Distilled Water	6.95	100.0

* Injections caused much pain and were followed by light muscular spasm in every case.

† Injections caused much pain and were followed by light muscular spasm in 5 out of 10 cases.

‡ Injections caused much pain and were followed by light muscular spasm in every case.

findings on the experimental control group we would be justified in anticipating degenerative changes in 15 per cent of the nerves of this series due to the effects of pressure produced by the injected solution.

Thus, the maximum effect of factors causing degenerative changes in this series extraneous to the chemical and physical (not pressure) characteristics of the injected solution would be 35 per cent, according to our findings. Since the incidence of degeneration in this series was 70 per cent we must attribute at least 35 per cent of the etiology of this condition to the character of the solution injected. That the injection solution accounts for a greater percentage of this pathosis is indicated by the character of the degenerative change, which in this series is remarkably constant in both quality and quantity.

A study of the nerves of the animals subjected to solutions of osmotic pressure values far more remote from the normal than in this series (right legs of animals 50-59), lead the writers to the conclusion that the degenerative changes described were not due primarily to the osmotic pressure value of the injected solution, but could be due to a combination of this factor with the low pH value (5.98), of the solution in question.

SUMMARY AND CONCLUSIONS

In an attempt to ascertain the importance of pH and osmotic pressure values in solutions used for local anesthetic purposes, an experiment was devised whereby the effect of each of these factors on the morphological character of the tissues receiving the injected solution could be tested separately.

The range of pH values of the solutions used in this investigation extended from 4.37 to 8.77; whereas the osmotic pressure values ranged from that of distilled water to 0.9 per cent NaCl solution.

From a consideration of the observations reported in this paper the writers were led to the following conclusions: 1) That the pH of a solution used for local anesthetic purposes, when this does not extend beyond 4.37 to 8.77, does not in itself cause any observable morphological alteration in peripheral nerve fibers of the cat. 2) That the osmotic pressure of a solution used for anesthetic purposes, when this extends between that of distilled water and 0.9 per cent NaCl solution, is not the primary causal factor of any observable morphological alteration in peripheral nerve fibers of the cat. 3) Although neither pH nor osmotic pressure values, within the ranges indicated, could in themselves be shown to be responsible for any of the described mor-

phological changes in peripheral nerve fibers, evidence was presented indicating that a combination of these 2 factors may be responsible for the morphological changes in the nerve structure reported in this paper.

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CLINICAL STUDY OF ARRESTING DENTAL CARIES

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A review of the literature bearing on dental caries suggests that more clinical investigation of this disease is necessary before we can expect much further progress. A clearer statement of what is meant by the term "dental caries" and a more adequate description of the various physical manifestations of the lesion are required. This presentation offers (1) a working clinical definition (2) a description of certain physical manifestations of dental caries and reports (3) the results following an experimental treatment of the disease.

For purposes of this presentation dental caries may be defined as necrosis and the accumulation of debris associated with cavitation on that surface of the tooth which is exposed in the oral cavity (Eliot, Souther, Anderson and Arnim, 1934). There is always cavition in clinically evident dental caries which has progressed beyond the earliest stage. Debris is present in the cavity owing to disintegration of the tooth itself and to oral accumulations. Necrosis of the organic substance of the tooth, as well as of the introduced particles, likewise occurs. These are the significant physical signs of dental caries. As soon as the lesion has involved the dentin and as long as the pulp of the tooth is still intact, there is the symptom of sensitiveness to physical or chemical irritants.

A criterion commonly employed in making a diagnosis of early dental caries is a "catch" with the explorer. This is not sufficient evidence that decay is actually present. The tip of an explorer will readily catch any deep pit or fissure where the enamel is intact, as well as where it is broken by decay. One should feel with the explorer a definite softness of necrotic dentin and often the gritting of the broken enamel edges instead of the resistance of hard enamel. If one can also elicit a local sensitiveness on pressure with the explorer, one can be more certain of the diagnosis of dental caries.

In a clinic where many previously untreated patients are encountered, one observes carious lesions that progress rapidly, slowly, or at varying intermediate rates. These lesions may correspond to acute and chronic processes observed in other diseases. Furthermore, patients may present carious lesions which apparently have become arrested.

Fig. 1 shows a mandibular left first permanent molar with a lesion which might be called acute dental caries. Although it involves most of the pits and fissures of the occlusal surface, there is still comparatively little loss of enamel. The white, opaque, fragile looking margin is typical of the undermined enamel bordering active caries. The cavity opening is small compared with the whole region of destruc-

FIG. 1

FIG. 2

FIG. 3

tion. The cavity contains loose, soft debris and in the deeper portions, a tough, leathery mass of decalcified dentin is encountered which offers more resistance to the passage of an explorer.

Fig. 2 shows a type of decay in which a greater proportion of enamel, compared with the dentin, is lost. A cavity of this form is not so favorable to the retention of the loose soft debris. The tough, leathery portion of the caries is exposed to view. This type of lesions seems to run a slower course and may be chronic in character.

Fig. 3 illustrates a lesion with still greater loss of enamel in proportion to the dentin than is shown in *figs. 1* and *2*. The enamel shows no undermining; instead of a white, opaque, fragile border, it presents a hard, rounded, translucent margin. The dentin, which is widely exposed, is relatively insensitive, contains no tough, leathery material, presents a hard, polished surface that will not permit the introduction

of an explorer, and shows no accumulation and retention of necrosis or debris. This may be an arrested condition.

Patients suitable to this study were selected from the Dental Clinic of the New Haven Hospital and Dispensary. The group included patients with no dental caries, those with typical dental caries of slight, moderate or marked degree, and those presenting the unusual manifestations of this lesion.

The routine of the study has been as follows, (1) general physical examination, (2) careful dental examination including, for the purpose of record, a photograph, roentgenograms, a plaster model, and a written description, (3) bacteriological studies of saliva, of debris on the surface of the teeth, of debris in the cavity, and of necrotic dentin, (4) physico-chemical studies of the tooth structures, and (5) histopathological studies of teeth with various manifestations of dental caries.

This report will concern principally the results obtained following the reëstablishment of the chewing function in carious teeth suffering from disuse, and accumulation and retention of food and other debris. The observations were made on a series of 40 patients. Molars of the type shown in *fig. 2* were selected for operative treatment which was performed on 20 permanent molar teeth in 5 males and 5 females. Their ages ranged from 10 to 20 years. Only 3 patients of this group had developmental defects of the enamel.

The treatment consisted in the removal of the projecting and overhanging enamel to the level of the solid dentin with the aid of a mallet and chisel. The decalcified and necrotic dentin was curretted away with a discoid excavator, only a thin layer of partially decalcified and stained dentin being left in situ to furnish temporary protection for the underlying sensitive dentin. The enamel margin was then smoothed over and the patient dismissed. *Fig. 4C* is a photograph of the tooth shown in *fig. 4B* following this treatment. In the more sensitive teeth the operation was performed in several stages with intervals of a few days or weeks. Follow-up observations have been made at regular intervals over a period from 6 months to 3 years.

All of the treated teeth, up to the present, have shown either partial or complete arrest of the carious process. The records of 2 of these

patients will be reviewed to illustrate typical changes that followed treatment.

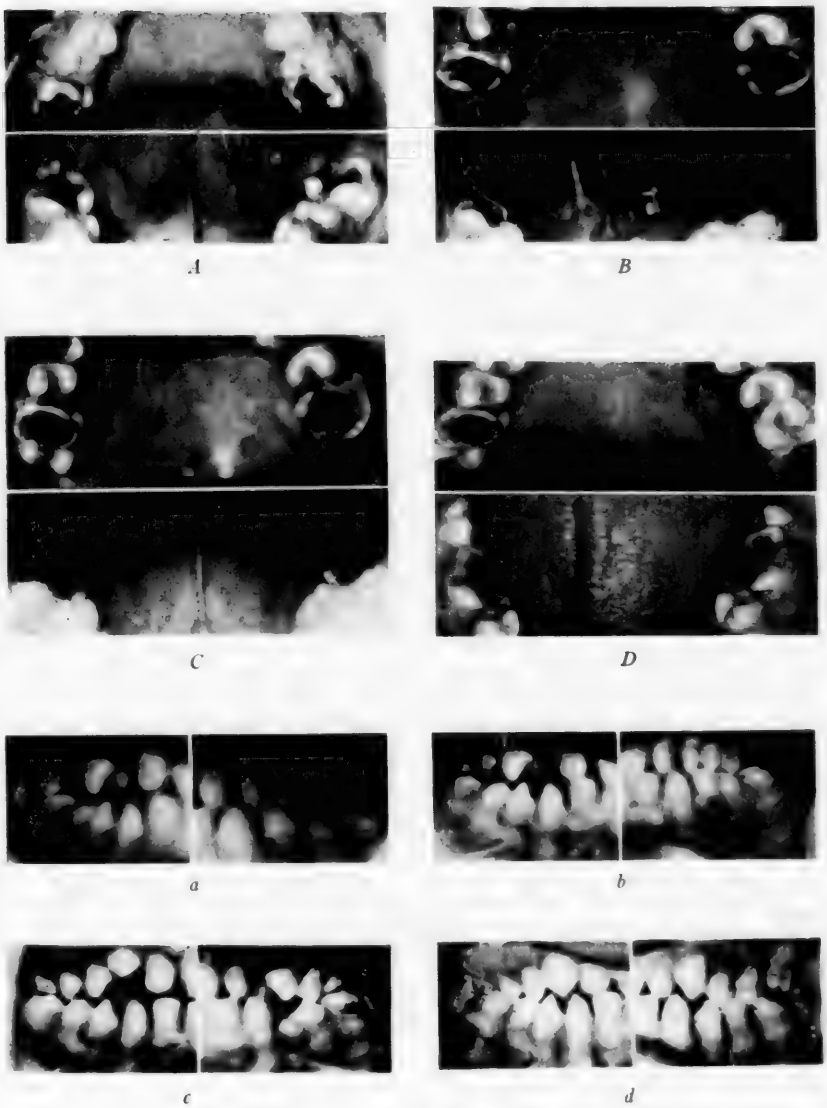
Case I, Hospital No. A 48295. Patient was a youth, 17 years of age, who came to the Dental Clinic with a complaint of pain in the left maxillary region. General physical examination was essentially negative. There was a markedly carious left maxillary first permanent molar with marginal gingivitis and acute and chronic infection of the tissues surrounding the remaining roots. The patient stated that for an indefinite period of time he had been chewing his food largely with teeth on the right side. The right mandibular second permanent premolar (*fig. 4A*) which was in active mastication although broken down on its chewing surface, was erupted into occlusal contact with its opponent. It presented an occlusal surface similar to that illustrated in *fig. 3*. Except for two small carious areas on the right maxillary molars and premolars, these teeth were in good functional order. The unopposed left mandibular first molar (*fig. 4B*), which was out of func-

A *B* *C*

FIG. 4

tion, presented chronic caries with projecting enamel walls, decalcified dentin in situ, slight retention of accumulated food and debris, and was sensitive to the point of an explorer that penetrated through to the solid dentin. This tooth was treated by surgery alone (*fig. 4C*). It was seen at regular intervals over a period of 2 years, during which time an attempt, only partially successful, was made to elevate it to the occlusal plane with the aid of an orthodontic appliance. During this time, the maxillary left permanent second molar drifted slightly anteriorly. The occlusal surface of the mandibular left first permanent molar remained practically in the same condition as it was immediately following the treatment. During the past year, however, since the removal of the orthodontic appliance, the patient has been able to chew with more comfort on this tooth, and there has resulted dark staining, diminished sensitivity, and hardening and polishing of its occlusal surface.

Case II, Hospital No. A 59246. Patient, a girl, 13 years of age, came to the clinic for treatment because of pain in the left maxillary first permanent molar. Physical examination was negative except for dental caries of the 4 first permanent molars and early pulp infection of the left maxillary first permanent molar. There was present also an accumulation of food and debris with minimal gingivitis on the left side, above and below. The caries of this tooth presented physical characteristics similar to those observed in the other 3 first permanent molars and as illustrated in *fig. 2*. It should be noted that the remaining partially undermined cusps of all of the first permanent molars (*fig. 5A*) were in occlusal



A *B*

C *D*

a *b*

c *d*

FIG. 5

contact (*fig. 5a*). These 4 teeth were all subjected to the treatment described (*fig. 4C*). *Figs. 5B and 5b* show the condition of these teeth after the undermined enamel and most of the decalcified layer of dentin had been removed. In order to avoid too rapid exposure of the dentin, the treatment was done in 3 stages, one week apart. *Figs. 5C and 5c* show the condition of these molars 3 months later when the patient returned for treatment of the left maxillary first permanent molar which developed an acute abscess. It should be noted that the treated teeth have erupted again into occlusal contact and that there is an increased accumulation of food debris, etc. around the teeth of the left side. The patient had confined her chewing mostly to the teeth on the right side where the gingivae appeared normal and the occlusal surfaces of the first molars, which were treated 4 months before, had become somewhat hardened. After a record was made of the condition described, the left maxillary first permanent molar was extracted and the patient dismissed. *Figs. 5D and 5d* illustrate the condition of these teeth 11 months after the first visit. It will be noted that the left maxillary second permanent molar has erupted into occlusal contact with the teeth of the opposing jaw, and has almost assumed the position formerly occupied by the maxillary first permanent molar. There was a minimal amount of food accumulation and debris around these teeth. The remaining first molars presented highly polished occlusal surfaces with stained relatively insensitive dentin and without further cavitation associated with necrosis and debris accumulation.

Numerous discussions concerning the etiology of dental caries are to be found in the literature. According to some investigators there are factors such as faulty tooth structure, faulty metabolism, or the failure of body fluids or local tissue to establish immunity in dental caries. Others have stressed the importance of the tooth environment in the oral cavity such as the rôle played by micro-organism, food deposits, or saliva in the production of dental caries. Most dentists assume that the use of the teeth in chewing food is essential for the maintenance of the health of these organs and instruct their patients to exercise the masticatory function on coarse food stuffs. Although exact scientific evidence is wanting to support the theory that vigorous mastication is essential to the maintenance of health of the teeth, this assumption is accepted for this study.

The literature also shows that confusion has arisen over the concept "arrested caries." The use of this term which is not adequately descriptive has been misleading. It has been particularly difficult to reconcile the meager descriptions of the lesions, as they appear in the literature, with the physical manifestations accompanying the changes that are observed. There is considerable variation in the rate of progress of caries as well as in the sensitivity of the dentin in different individuals. Crowns of teeth whose occlusal enamel is missing and in which the dentin presents a relatively insensitive stained

hard polished surface are seen more often in large clinics than in private offices. Many patients applying to the clinic for treatment have had no previous restoration or replacement of teeth but come to the clinic mainly for extractions. Conditions illustrated in *fig. 3* may occur spontaneously following undermining decay, crushing, and wearing away of enamel. Such teeth may continue to erupt into occlusal contact with their opponents. Patients usually show no disposition to favor these comparatively insensitive teeth and their use in mastication over a period of time may produce this smooth polished surface. The evidence and statements of all patients in this group, giving a reliable history, in whom such teeth were found, indicated that they were actively employed in the mastication of food. There was also evidence that protected portions of surfaces that favored accumulation and retention of debris may continue to decay or may again become carious at any time or any place where favorable conditions occur.

Black (1908) recommended cleaning the buccal surface in order to arrest decay in the enamel. He also suggested as a treatment for caries in sensitive children, cutting away most of the carious proximal surfaces in the deciduous molars, treating the exposed parts with silver nitrate and postponing the filling of the cavity to some later date. In a chapter dealing with the clinical form of the lesion, Kantorowicz (1925) described *stationare Karies*. According to his statement the condition is found almost exclusively on the chewing surfaces of the first molar and only in hypoplastic teeth. Colyer (1931) discusses the spontaneous arrest of dental caries and as a means of assisting the process recommended the following "stoning away any prominent enamel edges, which cause stagnation areas at their angle of junction with the dentin; the cavity must be non-retentive, and, as far as possible, part of the occlusal surface of the tooth, and the soft infected surface dentin removed; the exposed dentin is then sterilized by treating it several times with silver nitrate applications, an albuminate of silver is formed with what organic matter the silver salt comes into contact, and sensitiveness of the dentin, if present, is allayed." A number of investigators have stated that the changes called arrested caries are the result of a defensive mechanism on the part of the tooth which is stimulated to activity by the ingestion of a diet considered

more adequate with respect to certain elements. These are, respectively, vitamin C, (Hanke) vitamin D, and inorganic salts, (phosphorous and calcium), (Mellanby and Pattison 1928) a base-forming diet (Jones 1930) and a diet considered adequate in all respects (Boyd and Drain 1928). The reports of Boyd and Drain and Mellanby and Pattison on the occurrence of arrested caries in children on experimental diets revived interest in this subject. Other workers have since reported success in arresting dental caries by dietary means. However, the descriptions by all these workers of the carious lesions and changes that occur with the administration of experimental diets have been too indefinite. One must assume that the lesions which these authors called arrested caries have cavitation on a surface of the tooth which is exposed in the oral cavity and have no clinical evidence of accumulated debris and necrosis of tooth structure such as described above.

The mechanism by means of which the arrested carious changes observed in this study occur apparently are not dependent only upon the defense mechanism of the tooth itself, supposedly initiated by the ingestion of certain diets. Although it is recognized that the dentin, with the pulp intact, is a viable tissue and that changes may be seen in the density of this structure (Furrer 1922 and Beust 1934), no proof exists that such changes are brought about specifically by the ingestion of certain diets (Schour 1938). An important factor, producing the physical manifestations described above, is probably the mechanical removal of the parts that favor the accumulation and retention of necrosis and debris. Furthermore, it is possible that other factors in the oral cavity environment are altered by ingestion of certain types of food. The possibility that dental caries may cease to progress in protected pits, fissure or proximal surfaces is also admitted. Observations of such phenommena are as yet too few to include in this report. Although Boyd and Drain emphasized the importance of adequate diet, they, as well as others, state that sclerosis or hypercalcification of dentin is a defensive mechanism against decay. Practically all authors dealing with the nutritional aspect of the teeth express the belief, either directly or by implication, that a hypercalcified tooth is a desirable one since it forms a strong defense against the invasion of caries.

The assumption that calcium salts are the most important elements of the tooth and that the more of these inorganic substances that are laid down in the tooth the less likely it is to undergo decay is without scientific basis. The substance that is quantitively the greatest in the tooth may not be more important to the health of the organ than that which is the least in quantity. Furthermore, since the enamel, which is far harder than the dentin, decays so readily, it is difficult to see why one should attempt to make the dentin more like enamel in the hope that it will reduce decay. It has been shown experimentally by Benedict and Kanthak (1930) than an acid will attack the more mineralized part of the enamel, namely, the rods first, rather than the structure containing a larger amount of organic matter. This observation coincides with the accepted knowledge of the chemistry of such substances.

SUMMARY

Some of the various physical manifestations of dental caries have been described and discussed here from the clinical standpoint. It has been suggested that these lesions which progress at slow, rapid or intermediate rates or present arrested conditions show reactions comparable to acute, chronic, and healed stages of other disease lesions. The result of an experimental treatment of dental caries has been presented and the similarity between the experimentally arrested and the spontaneously arrested lesion has been pointed out.

The experimental arrest of dental caries followed the removal of the decay and those portions of the teeth that favored accumulation and retention of food and debris. Clinical evidence indicates that the masticatory function is important as an aid to the arrest or healing of dental caries.

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CONCERNING THE VITALITY OF THE CALCIFIED DENTAL TISSUES. II[1]

The Permeability of Enamel

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Previously reported studies (1) in vital staining have established the permeability of dentin and enamel. The passage of stain particles through dentin and enamel of dogs and young human beings was produced by inserting dyes into the dentin or pulp. The particles were transported by a fluid present in the dentin, termed by the authors the dental lymph, which has its origin in the pulp.

It is the purpose of this investigation to test the permeability of normal enamel of dogs and human beings from without-inward. The permeability of previously decalcified areas such as incipient caries is not under consideration. In the earlier report (1), the presence of potassium permanganate and other experimentally introduced dyes was demonstrated in the enamel rod sheaths penetrating by way of the dentin, illustrating the permeability of enamel from within-outward (*fig. 1*). The following report is the result of efforts to determine whether potassium permanganate, silver nitrate or methylene blue can penetrate normal enamel from without-inward.

A permeable membrane, generally speaking, permits the passage of substances in both directions, so that one may conceive of, not only the passage of dental lymph into the enamel from the interior, but also the passage of saliva into the enamel and dentin from the surface. Head (2), Pickerill (3), Bunting and Rickert (4), Fish (5), and others suggested that a possible post-eruptive calcification of enamel occurred through the agency of saliva. This was followed by attempts

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to "harden" enamel by the addition of calcifying substances to saliva. Andresen (6), Bodecker (7) and Wilson (8) reported favorably on this subject. Bodecker (7) believed that the mineral content of enamel may be increased from the interior, but in cases of rampant caries, Andresen's remineralizing powder might be used as a makeshift to superficially harden enamel. Bunting and Rickert (4) discussing the permeability of enamel, conclude that: "Enamel and dentin are porous . . . . . and admit the passage of salts and solvents of saliva to the pulp, and blood to the exterior." Fish (5), in staining vital teeth of a dog, used among other reagents, an aqueous solution of methylene blue under crowns. He observed that the stain persisted

FIG. 1. Section of maxillary second incisor of dog stained *in vivo* with $KMnO_4$ for 7 days. Dye placed in cavity prepared in dentin and sealed with dental cement. Dental lymph transported dye through tubules into enamel defining bands of Shreger.

on the surface of the enamel for several days and deduced that the enamel was permeable from without-inward. His report does not include the examination of sections showing actual penetration. Recently Kanner (9) reported that the enamel of adult human teeth is completely permeable in vivo to polychrome methylene blue, methylene blue and gentian violet, and that it is probable that the constituents of saliva normally penetrate the enamel to the dentino-enamel junction.

Leigh (10), Turner (11) and J. Leon Williams (12) opposed the theory that saliva entered the enamel. Williams questioned the physiological function alleged to take place in a living tooth as being identical with a chemical phenomenon occurring in a dead tooth out-

side of the mouth. Turner (11), in disagreeing with the theory of porosity in enamel, states, "Since horny epithelium is in all other parts of the body a protective layer, we may conclude that at least it was not nature's original plan to place any dependence on post-eruptive hardening of the enamel by means of lime salts derived from the saliva."

The addition of calcifying substances such as Andresen's remineralizing powder to the saliva showed beneficial clinical results in cases of rampant dental caries (2, 6, 7, 8). However, if normal enamel is likewise "porous," then it might readily follow that saliva would carry not only its calcium salts into the enamel but also other substances. It was reported that extracted human teeth immersed in saliva, stained more readily than those fixed in formalin (4). This suggested that teeth, in their normal environment, surrounded by saliva, were more permeable than those used for experimental purposes in the laboratory. Further tests showed the reason for this difference in permeability. In order to determine the scope of physiological reactions which may occur in vital teeth, it is necessary to compare their permeability in three different states i.e. vital teeth, extracted fixed teeth and extracted non-fixed teeth.

The permeability of vital teeth will be discussed after presenting evidence based on present observations. At this point a comparison of fixed and non-fixed teeth is necessary for the comprehension of much work that has been done on extracted teeth. The fundamental error made by many investigators, including one of the authors (C. F. B.), was to apply laboratory observations of stain penetration made on extracted teeth, to teeth containing vital pulps surrounded by saliva. It was thought that the removal of the teeth from the mouth would cause only negligible changes in the calcified tissues, the dentin, cementum and enamel. Hence it was considered permissible to apply observations made in the laboratory to vital teeth.

The permeability of any tissue depends greatly on the porosity of its channels. If these are open, then fluids can diffuse readily; if, on the other hand, they are clogged with a coagulant, then permeability is greatly reduced. This variation is clearly marked in fixed and non-fixed teeth. In order to comprehend this difference in permeability, it is necessary to mention the dental lymph. Its presence

has been established, although its exact chemical composition is unknown. Nevertheless, there is evidence that it contains traces of protein, based on the observation that fixed dentin shows remnants of a coagulant in the dentinal tubules. Inasmuch as fixing the teeth coagulates dental lymph, this procedure clogs all microscopic channels thus greatly reducing the permeability of the tissue. Consequently the rate and depth of stain penetration noted on fixed teeth is much less than that observed in vital teeth.

A difference in permeability has been defined also in vital teeth and in those that have been recently devitalized. It was observed that the permeability of the latter is temporarily increased, leading to the conclusion (1): "that certain physical and chemical changes which occur in dentin and enamel are dependent to a large extent on the vitality of the dental pulp and the state of the dental lymph."

All normally exposed surfaces of the body are derived from the ectoderm, resting upon a mesodermic tissue. The function of the epithelial covering is primarily protective and it is often referred to as an epithelial envelope. Enamel, a calcified epithelial structure, possesses a protective function similar to skin. A consideration of this similarity of function presents an interesting analogy. Discussing the permeability of skin, Starling (13) states, "It may be regarded as established that the uninjured skin is impermeable to watery solutions of salts and other substances, on the other hand it is possible to produce a certain amount of absorption of substances dissolved in fatty vehicles." Sollman (14) reports that the absorption of fatty substances occurs through the hair follicles, therefore we may consider it established that the epidermis is impermeable from without. The enamel, resting upon the dentin of mesodermic origin and receiving from it the dental lymph is comparable to the stratum corneum of the skin, being supplied by an interstitial fluid from the underlying mesodermic tissue. Both enamel and the stratum corneum are permeable from within but not permeable from without, classifying both tissues as uni-directional membranes (semi-permeable).

EXPERMENTAL PROCEDURE ON DOGS

Experiments were performed on the teeth of both dogs and human beings. The saliva of dogs was stained with potassium permanganate for a period of three weeks. A

crown, to which was attached a perforated receptacle (PR.), was cemented over both of the maxillary third molars of a dog just below Stenson's ducts (*fig. 2*). The receptacles were covered with a perforated cap held in place by a spring, making it possible to insert compressed potassium permanganate tablets three times a day. In this manner, the saliva was stained a purple brown color for the entire duration of the experiment.

A further variation of the test was to determine whether the dental lymph escaped from the enamel at the surface of the tooth. Crowns, containing dry, triturated potassium permanganate were cemented over the canine teeth. In one case, the enamel was clinically perfect. In a second, the incisal third of the enamel was superficially ground with a sand paper disk to remove the enamel cuticle; and in a third, the dentin was exposed at the incisal edge. A fourth was used as a control by cementing a crown, without potassium permanganate, over a normal tooth. The attachment of the crowns to the teeth was made by cement at the gingival margin only, so as to assure the direct contact of the dye with the incisal portion of the tooth.

RESULTS

Staining saliva in a dog by means of enclosing compressed potassium permanganate tablets in receptacles attached to the third molars resulted in staining the maxillary teeth. In the anterior region of the mouth, the teeth were practically unstained even though the saliva was definitely brownish purple. It was observed that those surfaces of the teeth that came in contact with the tongue, lips or food were practically unstained due to friction. *Fig. 3* is a photograph of the buccal surface of a maxillary second molar. This tooth like the other maxillary molars was stained mainly in the concavity of the buccal surface for the reason noted above. The stained teeth were sectioned to study the enamel microscopically, using oil instead of water to prevent distribution of the dye during grinding. *Fig. 4* is a longitudinal section of the same tooth shown in *fig. 3*. This shows that there was no penetration of the stain into the enamel. Sections of the other stained and unaffected teeth demonstrate the same result; i.e. no stain penetration into the enamel.

The second set of experiments, in which dry potassium permanganate was sealed over the enamel by means of crowns, show similar results. The incisal half of the teeth in contact with the dye were not stained, indicating the inability of dental lymph to pass beyond the surface of a tooth in adult dogs. On removal of the crowns, the potassium permanganate was dry and there was no surface stain except in those areas where the dye had come in contact with the oxyphosphate cement. It is highly significant that in this area there

FIG. 2

FIG. 3

FIG. 2. Crown with perforated receptacle (PR) used to stain saliva with potassium permanganate.

FIG. 3. Buccal surface of maxillary molar of dog after staining the saliva with $KMnO_4$ for three weeks. The concavity on the buccal surface of all maxillary molars was stained.

FIG. 4

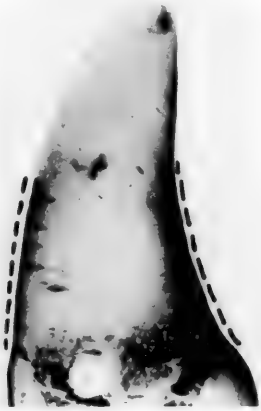

FIG. 5

FIG. 6

FIG. 4. Longitudinal section of the molar in *fig. 3* showing no penetration of stain into the enamel.

FIG. 5. Mesial surface of mandibular canine tooth after removal of crown containing dry $KMnO_4$ at incisal half. The cement was placed at the cervical half of the crown indicated by dotted line. Note staining of surface of enamel at area where the dental cement came in contact with dye. No surface staining at incisal half where dye without cement made contact with enamel.

FIG. 6. Longitudinal section of tooth in *fig. 5*. There was no penetration of dye at incisal half. At cervical half the $KMnO_4$ penetrated not only the enamel (E) but also the dentin (D).

was not only dye penetration through the enamel but also into the dentin. *Fig. 5* shows the mesial surface of a mandibular canine tooth which had been stained in this manner. The incisal half shows no effect of the stain whatever, whereas the cervical half is stained in that area where the dye came in contact with the cement attaching the crown to the tooth. *Fig. 6* is a longitudinal section of the same tooth. This demonstrates that there was no penetration of the dye into the incisal half of the enamel while at the cervical portion, where the dye came in contact with the dental cement there are several areas where the dye penetrated not only the enamel but also the dentin.

In one case the dentin was exposed at the incisal edge. In this case (*fig. 7*) it may be noted that the surface stain is similar to that shown in the previous illustration. On sectioning the tooth (*fig. 8*), it was noted that at the cervical half there was penetration of the stain into the enamel while the incisal half of the enamel was unstained. The exposed dentin in this plane demonstrates two strands of stained tubules. The study of changes in permeability of exposed dentin requires further investigation and may throw new light on the diffusion and behavior of dental lymph.

It must be made clear that these observations were made on the teeth of dogs. These have been shown to be more permeable than those of man, nevertheless, it seems worthwhile to perform similar experiments on human teeth to define whether dental cements partially decalcify the enamel. Should this be true, even to a slight extent, then the use of cement under orthodontic bands should be replaced by another medium or some reagent such as a varnish should be used to protect the enamel.

EXPERIMENTAL PROCEDURE ON HUMAN BEINGS

The experimental procedure on human beings consisted of attempts to introduce stains into the enamel from without by means of Andresen's remineralizing powder (6) to which potassium permanganate was added to the ratio of 1 to 4 by weight. This was applied to the teeth twice daily over a period of three weeks. Other human teeth were bathed in vivo with potassium permanganate, silver nitrate and methylene blue in aqueous solutions or, in a second set of experiments, dissolved in the saliva of the patient. The stains were applied under a rubber dam to the repeatedly moistened teeth for periods varying from ten minutes to two hours.

RESULTS

The above tests made on the teeth of man were similar to the results observed in the animal experiments. Observations showed that it was impossible to introduce the stains into *normal* human enamel from the surface of the tooth. Those teeth treated with Andresen's powder and potassium permanganate were unaffected on the surface or in the interior of the enamel, but salivary calculus, adherent to the tooth, did show a definite penetration of the stain.

FIG. 7

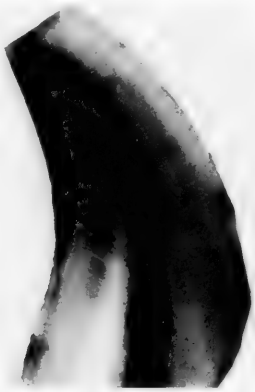

FIG. 8

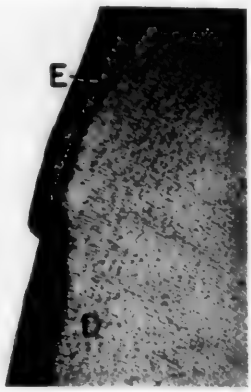

FIG. 9

FIG. 7. Mesial surface of mandibular canine of dog treated in the same manner as *fig. 5* with the exception of exposing dentin at incisal edge.

FIG. 8. Longitudinal section of same tooth (*fig. 7*) showing penetration of stain in enamel. At incisal edge two strands of tubules were stained illustrating changes in permeability of recently exposed dentin.

FIG. 9. Transverse section at cervix of human tooth stained in vivo with silver nitrate. Exposed dentin (D) stained at periphery, enamel (E) unstained.

Teeth treated in situ with silver nitrate and methylene blue showed surface staining only. In one case, a 10 per cent solution of silver nitrate was applied to the buccal and lingual surfaces. Twenty-four hours later the surface stain disappeared and the tooth resumed its normal appearance. This may be attributed to the friction caused by the tongue and cheeks as found in the animal experiment. The tooth was extracted for examination immediately following a second application of silver nitrate. The only areas that took the stain

were the exposed cementum at the cervix of the tooth as well as carious and abraded dentin. *Fig. 9* is a photomicrograph of a transverse section at the cervix of a human tooth stained with silver nitrate. It can be noted, that while the enamel did not take up the stain, the superficial layer of eroded dentin was deeply stained. However, the stain particles were not carried through the tubules to the pulp, but were limited to the area of dentin at the periphery. It was evident

FIG. 10. Transverse section of human tooth stained with $AgNO_3$. Crack (C) extending into dentin stained with dye. Lamella (L) not stained.

that the dentinal tubules of this area had become blocked due to the formation of secondary dentin and the metamorphosis of the dental lymph, thereby preventing the passage of stain particles to the pulp.

One interesting observation was made illustrating the difference between lamellae and cracks in the enamel. They are remarkably similar. Churchill (15) differentiates cracks from lamellae; he found that the former extend into the dentin. Observations of teeth

stained in vivo confirm this opinion. Leigh (10) made the same observation staining teeth in vitro. Those structures having the characteristics of lamellae did not take the stain, whereas the dye penetrated the cracks through the enamel and dentin. In *fig. 10*, a transverse section of a vitally stained human tooth, a crack and lamellae are demonstrated in the same specimen. Evidence of the presence of the dye was found in the crack (C) and not in the lamella (L).

The results of these observations seem to be at variance with clinical experience. The use of silver nitrate in the mouth by clinicians often results in the staining of the teeth. However, the areas that are stained must be carefully defined. Either they are: 1) at the cervices or the cementum; 2) on the dentin from which the enamel has been abraded; or 3) incipient carious lesions in which the enamel has been partially decalcified. It is clear that silver nitrate as well as other dyes can penetrate such areas.

The purpose of this investigation was to determine whether *normal adult* dental enamel is permeable from without; the results indicate a negative answer.

SUMMARY

Evidence has been advanced that stains can penetrate normal enamel from within, through the agency of the dental lymph but not from the surface of the tooth. Only as a result of certain changes such as incipient carious lesions in human teeth or the changes caused by the action of dental cement on the enamel of dogs does the enamel become permeable from without-inward. This may suggest that dental cements have a similar decalcifying effect on the enamel under orthodontic bands, crowns, etc.; a subject which is being investigated. The action of dental cement on the surface of the enamel in dogs appears to break down the surface impermeability, permitting stain particles to be carried into the enamel and dentin by the dental lymph. The stain was also demonstrated in cracks in human enamel but these are considered abnormal. It appears that enamel possesses the function of limiting the dental lymph to the tooth thus preventing its escape, just as the epidermis prevents the exudation of blood lymph.

Normal enamel seems to possess a protective function similar to that of skin; in teeth of dogs and young persons it is permeable from

the dentino-enamel junction up to the outermost zone of the enamel but not from without-inward, thereby classifying enamel as a unidirectional permeable membrane. The establishment of the impermeability of enamel from without-inward seems to preclude the possibility of post-eruptive calcification of normal enamel by means of a deposition of mineral salts present in the saliva.

Grateful acknowledgement is made to Dr. E. Zegarelli and Dr. Daniel E. Ziskin for valuable assistance in the clinical phase of this work. The co-operation of patients in permitting the experimental work previous to the necessary removal of the teeth is greatly appreciated.

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METHOD FOR DETERMINING THE SPECIFIC GRAVITY OF DENTIN AND ITS APPLICATION TO PERMANENT AND DECIDUOUS TEETH

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Measurements of the specific gravity of dentin date back at least to 1870 (1) and the methods employed may be classified as follows: 1. Water or mercury displacement (1, 3, 6). 2. Flotation (4, 5, 7). 3. X-ray densitometry (2). 4. Refractive index (5). These methods were not satisfactory for the study of a large number of deciduous and permanent teeth showing varying degrees of root resorption. A method was needed which would meet the following criteria: (a) give the direct mass: volume relationship; (b) permit the study of coronal dentin in deciduous teeth with resorption of the roots; (c) leave the specimens undamaged, available for further studies as to structure or chemical composition; (d) permit isolated observation or the comparison of large numbers of specimens; (e) give values representing the average specific gravity for the dentin of each sample.

The following method, which meets the above specifications with suitable accuracy, was devised. Essentially, it consists in preparing a tooth section of convenient thickness (approximately 0.34 mm.) and measuring its area, thickness and weight. From these values and the previously determined specific gravity of enamel, the specific gravity of dentin can be calculated.

METHOD

I. Tooth slabs were made by cutting through the long axis of the tooth with a $\frac{1}{4}$-inch dental saw and grinding them plano-parallel to about 0.34 mm. (0.01 mm.) on a special

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lathe. Both of these operations were performed under jets of cold water and the slabs stored subsequently in a formalin water solution. The thickness was measured by taking 6 to 10 micrometer readings at various points on the slab and using the average value.

II. The area of each section was found by making an enlarged photograph of it, measuring the area of the image with a planimeter, and dividing this value by the square of the enlargement ratio. Each slab was placed on a glass slide, laid in the film holder of the Leitz Valoy photographic enlarger and the image projected on white paper. Sharp

TABLE I

Enlargement ratios

MAXIMUM DIAMETER OF TOOTH SLABS	MAXIMUM DIAMETER OF SHADOWGRAPH	RATIO
mm.	*mm.*	
8.27	82.7	10.00
9.42	94.0	9.97
9.19	91.8	10.00
8.33	83.5	10.00
9.75	98.5	10.10
15.55	155.4	10.00
9.54	95.4	10.00
7.95	79.5	10.00
8.87	89.0	10.02
6.37	64.0	10.04
6.40	64.0	10.00
12.59	126.0	10.00
8.43	84.1	9.98
9.02	90.0	9.98
15.25	151.0	9.92
7.89	78.6	9.96
8.55	85.5	10.00
8.14	81.2	9.97
8.57	85.7	10.00
6.70	67.0	10.00
11.05	110.5	10.00
Average.....		10.00 ±.02

focus was obtained with a linear enlargement of $1:\sqrt{40}$ for permanent teeth and $1:\sqrt{100}$ for the smaller deciduous teeth, these sizes being convenient for 4 x 6 inch printing paper. The enlarger was left in this fixed position which gave an enlarging ratio quite constant for any group of teeth. This constancy is shown in Table I which gives the ratios for one group of 21 teeth. The projections were made on contrast, 4 x 6 inch, sensitized paper, and the resulting *shadowgraphs* were developed in ordinary developer (*fig. 1*).

III. The shadowgraph was dried, attached to a drawing board and the areas of dentin, enamel and the whole section were measured with a planimeter. This measurement was reproducible with an error of less than one per cent.

IV. Following immersion for 24 hours in distilled water, the slabs were blotted to

remove all visible water. Then each was weighed separately in a wide mouthed glass-stoppered bottle of about 1 or 2 cc. capacity, using an ordinary analytical balance with calibrated weights.

Earlier studies had shown the specific gravity of moist enamel to be essentially constant with a value of 2.95 by the immersion method. This is in agreement with values

FIG. 1. Shadowgraph of tooth section

TABLE II

Results of repeated measurements on same tooth

TOOTH NO.	FIRST VALUE	SECOND VALUE	THIRD VALUE	AVERAGE VALUE
10-1	1.85	1.88	1.88	1.87
10-2	1.97	1.99	2.00	1.98
16-1	1.90	1.91	1.91	1.91
53-1	1.99	1.99	1.98	1.99
59-3	2.02	2.01	2.03	2.02
59-4	1.94	1.98	1.93	1.95

obtained with the very accurate methods of Manly (5) and Thewlis (4). Assuming that this value is constant, the density of the dentin was found by substituting in the following formula: $dD = \frac{\text{wt D}}{\text{vD}} = \frac{\text{wt slab} - 2.95\ \text{vE}}{\text{vD}}$ where vE is volume of enamel, vD is volume of dentin, wt D is weight of dentin and dD is density of dentin

V. To check the reproducibility of the method, 18 slabs were carried through all the steps, each set of measurements of thickness, weight, area, and enlarging ratio being repeated three times and on different days. A sample of the density values obtained are given in Table II. Corresponding values in columns 2, 3, and 4 are duplicates.

RESULTS

The average specific gravity of 191 slabs from permanent, noncarious teeth was 2.00 with an average deviation in density of 0.06, ranging from 1.71 to 2.23. This is somewhat lower than Black's

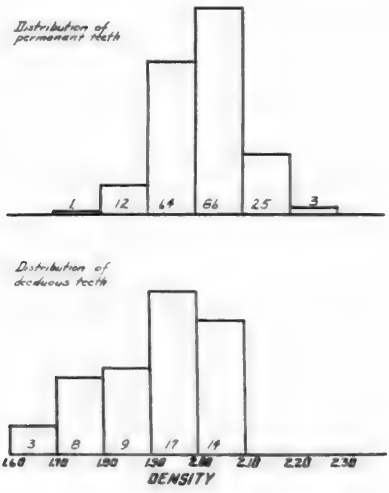

FIG. 2. Distribution of permanent and deciduous teeth along density axis. Values in columns are numbers of teeth. Curve drawn through these group averages has its maximum and limits for permanent teeth farther to right, both giving evidence of greater density. Slope is greater on both sides of maximum, indicating less variability of density in permanent teeth. Note that no values were found above 2.10 for deciduous teeth.

(3) value of 2.09 and Manly's (5) value of 2.14, probably because water and protein in the tubules and some cementum were included in the volume measurements.

The average specific gravity of 51 deciduous teeth showing no cavities was 1.91 with an average deviation of 0.10 and ranging from 1.64 to 2.09. In most of these teeth the roots were completely resorbed so that this value is for coronal dentin and may, therefore be slightly higher than an average value for whole teeth. However,

there is shown a significant difference between the specific gravity of permanent and deciduous teeth. *Fig. 2* shows the distribution of both permanent and deciduous teeth in different density groups from 1.60 to 2.30. Very little data from deciduous teeth are available for comparison with these results. Black (3) found a specific gravity of 2.07 for one deciduous root and Wedl (1) reported values of 1.19 to 2.17 for 4 whole deciduous teeth which values, of course, are not comparable with ours.

SUMMARY

The described method for determining the specific gravity of dentin has certain advantages, namely: 1. It is applicable to a large number of teeth. 2. There is no sacrifice of the tooth slab. 3. The values obtained by it agree with recent, very accurate methods. 4. It can be used for coronal dentin in deciduous teeth.

Values were obtained for the density of 191 permanent, non-carious teeth and 51 deciduous non-carious teeth with averages of 2.00 and 1.91, respectively.

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THE BACTERIAL FLORA IN DIFFERENT PARTS OF THE MOUTH[1]

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In comparing the bacterial flora of different mouths (Bibby, 1938b) it was noticed that the variations between different parts of one mouth seemed more definite than the differences occurring between similar sites in different mouths. This observation is in keeping with the general idea that bacterial flora in various parts of the mouth is not the same. However, an investigation of the literature shows that little positive evidence has been published to support this view. Pickerill and Champtaloup (1913) reported that Gram-positive cocci were more common in the saliva than in material from the gingiva, whereas Gram-negative cocci, bacilli, fusiform organisms and filaments were more plentiful in the latter site. Brailovsky-Lounkevitch (1915) found that while Gram-positive diplococci predominated in smears from the cheeks, tongue and palate, other organisms, such as Gram-negative bacilli, fusiform bacilli and filaments, were more numerous on the gingiva and between the teeth. It has been noted by Kostecka (1924) and Andre (1934) that the obligate anaerobes (streptothrix, vibrios and spirochetes) were found only in association with the gingival crevices. In this paper the bacterial flora in different areas of the mouth are compared.

METHODS

The previously described method (Bibby, 1938a) in which the percentages of morphologically distinct groups of microörganisms are estimated by direct microscope examination of smears was used in obtaining the results presented here. In obtaining material for study, the technique followed was essentially the same as that previously described (Bibby, 1938b). It was modified only for special purposes. So that smears from corresponding areas in different mouths would be from the same sites, so far as possible, the carefully regulated procedures described in a previous paper (Bibby, 1938b) were fol-

[1] The study upon which this report is based was made possible by funds granted by the Carnegie Corporation of New York.

lowed. In collecting the specimens the same instruments were applied as nearly as possible in the same manner in all mouths.

Examinations were made of material from over 70 different mouths. The same grouping of organisms was employed as that used in the previously mentioned paper. The original plan of the study called for only 1 smear from a given area in each mouth, but some of the larger series of counts include 2 or more smears taken from the same mouth during different studies.

FINDINGS

Estimates of the percentage distribution of the groups of microorganisms revealed considerable differences in smears taken from the same areas in different mouths. Since a detailed presentation of the results serves no useful purpose they are summarized in Table I which shows the average percentages and the highest and lowest percentage of each group of organisms found at the particular sites. An examination of the table shows that wide variations in the percentages of bacterial groups occurred in all series of counts. Although the variations in a single group sometimes reach a magnitude of almost 100 per cent there was generally some grouping of figures in the middle with only a few falling at the extremes. For example, in the series of counts of mouth washings 75 per cent of the percentages of the various organisms fell within much narrower ranges. For Gram-positive cocci the percentage range was 38.4–60.6; for Gram-negative cocci 18.7–36.7; for Gram-positive bacilli 0–4.9; for Gram-negative bacilli 1.1–9.3; for Gram-positive filaments 0–5.9; and for Gram-negative bacilli 1.4–9.1. This grouping was equally as marked in counts from the other sites.

Comparisons reveal only slight or indefinite differences between smears from various parts of the mouth. The mouth washings have a high percentage of Gram-positive cocci but otherwise an average distribution of organisms. The smears from the posterior surfaces of lower molars are characterized by the highest percentage of filamentous organisms, the Gram-positive types being more numerous than elsewhere and the Gram-negative types being out-numbered only by one other area. A low percentage of Gram-positive bacilli and a high occurrence of Gram-negative filaments distinguishes the occlusal

fissures. The buccal surfaces of upper molars show large numbers of filaments especially the Gram-negative forms which were more frequent here than elsewhere. The highest percentage of Gram-

TABLE I

Variations in percentage occurrence of groups of microorganisms in different parts of the mouth

SITE	NO. OF SMEARS		PERCENTAGES OF TOTAL ORGANISMS COUNTED					
			Cocci		Bacilli		Filaments	
			Gram +	Gram −	Gram +	Gram −	Gram +	Gram −
Mouth Washings	36	Av.	48.6	28.4	4.4	6.8	4.6	7.1
		Range	1.8–71.2	0–65.4	0–31.2	0–52.7	0–9.9	0–20.6
Posterior Surfaces of Lower Molars	37	Av.	36.7	29.6	5.2	8.6	6.8	13.0
		Range	7.0–55.6	0–57.4	0–24.9	1.5–50.6	0–17.5	3.6–55.0
Occlusal Fissures	47	Av.	36.4	36.5	1.7	8.2	4.3	12.9
		Range	14.1–68.7	5.0–70.0	0–24.5	0–38.9	0–20.9	3.6–47.1
Buccal Surface, Upper Molars	18	Av.	28.6	40.1	3.7	7.7	3.8	15.5
		Range	16.3–47.0	9.0–59.1	0–21.2	0–22.6	0–10.7	6.0–32.8
Buccal Surface, Lower Molars	11	Av.	32.7	38.1	4.9	19.1	0.5	4.9
		Range	4.9–56.4	0–70.9	0–19.0	2.6–55.7	0–3.1	0–20.0
Mucous Film, Lower Molars	6	Av.	39.5	32.4	1.7	21.1	0.2	5.0
		Range	5.1–63.0	8.2–61.8	0–8.4	1.8–60.4	0–1.0	0–11.1
Buccal Mucosa	25	Av.	53.1	30.3	3.4	4.6	2.8	6.2
		Range	0–95.5	0–82.5	0–33.3	0–21.2	0–10.2	0–23.8
Gingival Margins	22	Av.	36.3	29.9	8.1	10.6	3.9	9.5
		Range	17.8–63.5	0–67.1	0–35.0	0–30.7	0–13.1	2.2–28.7
Pyorrhoea Pockets	6	Av.	37.7	35.7	4.9	6.1	4.8	10.4
		Range	16.8–65.8	14.6–65.7	0–12.5	2.4–10.5	1.2–7.8	2.2–20.5
Cavities	68	Av.	36.5	40.2	5.1	7.7	4.7	5.7
		Range	15.1–55.6	20.0–85.2	0–16.8	1.5–14.0	0–17.5	0–18.6

positive cocci and total cocci is encountered in preparations from the buccal mucosa. Smears from the gingival margins are characterized only by a relatively high percentage of Gram-positive granular fusi-

form bacilli. The carious cavities are characterized only by slightly more Gram-negative cocci than found elsewhere, and a resultant high number of total cocci.

An interesting finding is that the percentages of Gram-negative cocci are relatively high in smears from all sites and in the cavities are actually the most numerous group of organisms. In contrast the percentages of Gram-positive bacilli are consistently low indicating that they are not prominent constituents of the oral flora.

DISCUSSION

The results of this study do not establish significant differences between the flora of various parts of the mouth. None of the observed differences are sufficiently constant to enable the source of a smear to be determined by a bacterial examination. In general our findings support the conclusions of previous workers (Pickerill and Champtaloup, 1913, Brailovsky-Lounkevitch, 1915, Kostecka, 1924, and Andre, 1934) in that they show larger percentages of pleomorphic fusiform and filamentous organisms in the gingival regions, and areas where bacterial accumulation can take place, than are found on the mucosa, the superficial film on teeth or in mouth washings, in which saliva and mucous secretions are important constituents. Our findings may be interpreted to suggest that Gram-positive cocci proliferate on the buccal mucosa and filamentous types in the gingival crevices and areas undisturbed by the washing action of saliva. The numbers of cocci or filaments in different areas may be traced to the predominance of one or the other of these influences. In view of the prominence which has been given to the lactobacilli as a cause of dental caries it is surprising to find that organisms morphologically resembling that group are not more prominent in carious cavities. This is especially true since the percentages given above for Gram-positive bacilli represent certain bacillary elements which in no circumstances could be considered lactobacilli. The high percentages of Gram-negative cocci occurring in all sites and especially in the cavities is an unexpected finding which it will be interesting to verify in other studies.

In spite of the apparent failure to demonstrate distinctive flora in various parts of the mouth it is still felt that there may be more definite differences than are apparent from this work. Before the occur-

rence of such differences can be denied it is necessary to take into account several possibilities which appeared as this study progressed. *First*, a classification of organisms must be used which distinguishes more satisfactorily the types of organisms encountered and takes advantage of morphological details which can be established only by correlated morphological and cultural studies. *Second*, methods of collecting material must be devised which will exclude more effectively organisms from adjacent parts. Since by our methods it was impossible to obtain material from the bottom of a fissure without including microörganisms from the superficial part, or to collect samples from the depth of a gingival crevice without the inclusion of extraneous material from the necks of the teeth, it can readily be appreciated that our smears cannot be considered as uncontaminated specimens. *Third*, a more definite and detailed subdivision of clinical oral conditions is necessary to avoid confusion resulting from comparisons of specimens which are fundamentally dissimilar although taken from the same site. *Finally*, the possibility of daily or weekly variations in the flora of one area has to be considered. When these short-comings can be eliminated it is felt that a study of this type may succeed in establishing the basic characteristics of the flora in different parts of the mouth. A subsequent paper reports progress in this direction.

SUMMARY

In 276 smear counts a comparison was made of the percentage occurrence of basic morphological groups of bacteria occurring in material obtained from 10 different parts of the mouth.

Little uniformity was found in the distribution of organisms in any of the individual areas studied.

Marked or constant differences could not be established between the flora in different sites, but increased percentages of one or another group of organisms were sometimes noted.

It is suggested that it might be possible to establish more definite differences if a more detailed classification of organisms was used, if samples from different sites could be separated more completely, if a more exact clinical differentiation of mouths was possible, and if probable variations occurring in flora from time to time could be taken into account.

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STUDIES CONCERNING THE ORAL CAVITY AND SALIVA

IV. Calcium (1) Calcium Content of Resting and Activated Saliva of Children[1, 2]

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A general interest in the subject of saliva dates from antiquity. Hippocrates 450 B.C. (63) wrote concerning the properties of saliva in various diseases. Later this interest became more specialized as shown in the review of salivary studies by Wright in 1841 (87). In reviewing the literature on saliva one is impressed by the large number of studies concerning the calcium content. The observations of earlier studies though qualitative in nature, were not essentially different from accepted findings today. Notable in this respect is the work of John Hunter, 1778 (42) and of Thomas Bell, 1830 (6), wherein they set forth their opinions with regard to the rôle of calcium in calculus formation. Table I has been prepared to show the numerous relationships studied in former salivary calcium studies. The table gives the various relationships along with the reference numbers to the different studies.

In analyzing the results of former salivary calcium studies there is apparent a definite lack of agreement in the means and ranges reported for both the resting and activated saliva (Tables VII, VIII, and IX). These differences are probably mainly due to the use of methods of different degrees of precision, and to the failure to deproteinize the saliva before employing what would ordinarily be an accurate and reliable method. Becks (5) has made an extensive study of former salivary calcium studies and has discussed in detail

[1] Read at the 16th General Meeting of the International Association for Dental Research, Minneapolis, Minnesota, March 12–13, 1938.

[2] This work was supported by a grant from the Beechnut Packing Company.

the advantages and disadvantages of the various methods employed for calcium work.

If salivary calcium is to be of any value as a diagnostic tool or in correlation studies it is imperative that a normal physiologic range

TABLE I

Former salivary calcium studies with reference numbers and relationships

DENTAL CARIES	COMPOSITION	DIET	METABOLISM
6, 9, 10, 13, 14, 15, 21, 22, 26, 27, 29, 32, 34, 35, 36, 37, 38, 40, 44, 46, 48, 49, 53, 54, 56, 57, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 82, 83, 84, 86	4, 5, 12, 16, 17, 18, 20, 23, 24, 25, 28, 29, 40, 43, 45, 46, 44, 48, 53, 54, 56, 57, 58, 60, 61, 62, 64, 66, 77, 78, 84, 86	14, 18, 33, 34, 35, 36, 37, 38, 53, 54, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76	1, 2, 6, 9, 10, 11, 33, 34, 35, 36, 37, 39, 52, 63, 67, 68, 69, 70, 71, 72, 73, 74, 83
METHODS	**SOLUBILITY**	**BLOOD**	**VARIATION**
5, 9, 13, 16, 17, 19, 31, 38, 46, 50, 51, 55, 60, 61, 79, 82, 85	7, 10, 15, 27, 29, 32, 47, 48, 49, 65, 69, 70, 71	18, 20, 26, 33, 34, 35, 36, 37, 38, 41, 46, 75	4, 17, 21, 38, 40, 41, 61, 77, 79, 82
CALCULUS	**RATE OF FLOW**	**PYORRHEA**	**FEEDING Ca SALTS**
4, 6, 14, 39, 42, 49, 65, 75, 77, 81	3, 12, 20, 23, 40, 41, 45, 66, 80	5, 6, 33, 34, 35, 36, 37	14, 20, 21, 45, 64, 82
PREGNANCY	**SYSTEMIC DISEASE**	**STIMULI**	**BACTERIA**
4, 13, 14, 54, 75, 82	21, 38, 54, 64, 77, 80	3, 20, 38, 45, 66	27, 30, 31, 65
AGE	**WATER CONTENT**	**ADSORBENTS**	**IRRADIATION**
4, 39, 64	66, 76	4, 58	26, 67
SEX	**PROTEIN**	**FASTING**	**COLOR OF TEETH**
38	17	17	13

and average be established. In establishing such a standard consideration must be given to age, sex, time of day and any other factors which might be responsible for variations. It was with these various facts in mind that the study herein reported was instituted.

METHOD

Selection of Subjects: A group of 819 children ranging in age from three to eighteen years was selected from 4 orphanages, 3 public schools and 1 industrial school. The children were all apparently normal healthy individuals. No physical examinations were given. However care was taken to exclude from the group any children who gave any recent history of illness or who had any symptoms of illness at the time of the examinations.

Procedure for making salivary calcium determination: The Clark-Collip (19) modification of the Kramer-Tisdall (50, 51) method for serum calcium was selected for the present study because of its precision (error of ± 3 per cent), moderate time requirement (approximately one hour including the time for preliminary treatment of saliva) and the comparatively small amount of saliva required (2–3 cc. or the equivalent of 0.2 mg of calcium). The procedure including the collection and preliminary treatment of sample is as follows: *A. Collection of salivary samples.* All samples were collected during the morning, at least one hour after breakfast. Two samples were collected, one a resting sample (i.e. one collected without the use of a stimulant of any kind other than normal physiological stimuli), the other, an activated sample (i.e. one collected with the use of a stimulant other than the normal physiological stimuli). For the collection of the resting sample the subject was kept as quiet as possible, usually in the seated position. Instructions were given to allow the saliva to flow into the mouth without unnecessary movement of the jaws or tongue, except to expectorate into a funnel and test tube provided for the collection of the sample. For the activated sample the subject is given a piece of paraffin approximately one gram in weight, and instructed to chew it for five minutes before starting the collection. The paraffin is chewed during the entire collection of the sample. The time required for the collection of both samples is taken in order to calculate the rate of flow. Six cubic centimeters of each type of saliva were collected.

B. Preliminary treatment of samples. 1. Removal of suspended material. Immediately after collection the tube containing salivary sample is stoppered, shaken thoroughly and allowed to stand for at least one hour or more before the calcium determination is made.

This treatment of the sample reduces the viscosity and facilitates the settling of the suspended material (cells, undissolved mucin, debris etc.) to the bottom of the tube. The relatively clear supernatant fluid can then be pipetted without the preliminary centrifugation step suggested by Becks (5) and Wainwright (85), which results in the loss of a small amount of calcium (17). However, if the determination is to be made immediately after collection, preliminary centrifugation must be resorted to in order to remove the suspended material.

2. Deproteinization. Due to the interference by protein in the calcium determination it is necessary to remove the salivary protein before proceeding with the actual determination (5), (26), (38), (55), (85), (86). By means of a pipette, 4 cc. of the supernatant saliva is withdrawn and placed in a centrifuge tube (care being taken not to disturb the sediment). To the saliva 4 cc. of 10 per cent trichloracetic acid is added, stirred with a glass rod and allowed to stand for 10 minutes. The mixture is then centrifuged for 5 minutes at 1500 R.P.M. The supernatant fluid is decanted and, saved for the calcium determination.

C. Precipitation of the calcium as oxalate. Four cc. of deproteinized saliva is placed in a 15 cc. graduated centrifuge tube measuring 6–7 mm. at the 1 cc. mark. Two cc. of a saturated solution of ammonium oxalate (4.2 per cent) is added. The reaction of the solution is adjusted to a pH of 5.9 by adding a drop of 0.04 per cent brom-cresol-purple indicator solution, then concentrated ammonium hydroxide drop by drop until a lavender-gray color persists after shaking the mixture thoroughly. If too much ammonium hydroxide is added and the solution is a purple color instead of a lavender-gray color 0.1 N HCl is added drop by drop until the mixture becomes lavender-gray. The mixture is allowed to stand one-half hour for the complete precipitation of the oxalate and is then centrifuged 10 minutes at 1500 R.P.M. The supernatant fluid is carefully poured off so as not to disturb the precipitate; while the tube is still inverted place it in a test tube rack for 5 minutes to drain. Drainage is facilitated by placing the rim of the tube on a pad of bibulous or filter paper.

D. Washing the precipitate. The precipitate is mixed with 3 cc. of dilute ammonia (2 per cent). The sides of the tube are washed when adding the ammonia. It is centrifuged for 5 minutes at 1500 R.P.M.,

the supernatant fluid carefully poured off and drained for 5 minutes as before. Washing is repeated.

E. Solution of oxalate in H_2SO_4. Two cc. of 1 N H_2SO_4 is added in such a way as to wash the sides of the tube and at the same time break up the mat formed by the precipitate. The tube is placed in boiling water bath for 1 minute before titration.

F. Titration with $KMnO_4$. The titration is made with a 0.01 N $KMnO_4$ solution, using a microburette to deliver the $KMnO_4$. The $KMnO_4$ is added drop by drop until a pink color is obtained which persists for 1 minute. (The $KMnO_4$ 0.01 N is made up fresh every day by diluting 5 cc. of 0.1 N $KMnO_4$ to 50 cc. with distilled water. The normalcy of the solution is checked against a standard solution of 0.01 N sodium oxalate. The 0.1 N $KMnO_4$ solution is made up fresh once a month and stored in a dark colored bottle in the ice box. [For preparation of solutions consult (19), (50), (51).])

G. Blank. The following blank is subtracted from the titration figure: No. of cc. of 0.01 N $KMnO_4$ necessary to titrate 2 cc. of trichloracetic acid treated according to the method outlined.

H. Calculation. The calculation for the determination is made according to the following formula: $\frac{(fy - b) \times 0.2 \times 100}{X}$ = mg Ca per 100 cc. sample.

f = factor of $KMnO_4$ $\frac{\text{(cc. of 0.01 N)}}{\text{cc. of KNn0}_4}$

y = number of cc. of 0.01 N $KMnO_4$ used in titration of sample.

b = number of cc. of 0.01 N $KMnO_4$ used in titration of blank.

X = number of cc. of sample used.

0.2 = mg of Ca in 1 cc. of oxalate solution.

I. Test Solution. The test solution used was made by dissolving 0.250 grams $CaCO_3$ in 1 liter of distilled water. This solution contains 10 mg of calcium per 100 cc. of solution. The method outlined above when used on the test solution gave a deviation from the actual amount of calcium present of from + 3 per cent to − 5 per cent with an average deviation of ± 3 per cent.

RESULTS

The discrepancy between the original number of cases (819) and the total number of cases reported (794) is accounted for by reporting only

those cases which had determinations made on both the resting and activated saliva (i.e. neither sample was lost through accident).

Tabulated in Table II are the means, standard deviations, standard error of the mean and ranges for the various age groups studied.[3] The

TABLE II

Means, standard deviations, standard errors, and ranges of calcium content of resting and activated saliva. (Males, females and both for various age groups.)

AGE GROUP	SEX	CASES	RESTING SALIVA MGS CALCIUM PER 100 CC.				ACTIVATED SALIVA MGS CALCIUM PER 100 CC.			
			Mean	S.D.	S.E.	Range	Mean	S.D.	S.E.	Range
yrs.										
1–6	M	4	6.10	0.60	0.30	5.5– 6.8	4.75	0.52	0.26	4.2– 5.4
	F	4	6.32	0.86	0.43	5.1– 7.1	5.47	1.13	0.57	4.2– 6.7
	B	8	6.21	0.70	0.24	5.1– 7.1	5.11	0.81	0.29	4.2– 6.7
6–10	M	112	6.77	1.04	0.10	4.0– 9.1	6.06	0.97	0.09	3.5– 7.8
	F	81	7.06	0.68	0.08	5.0– 8.5	6.43	0.65	0.07	4.5– 7.9
	B	193	6.89	0.90	0.06	4.0– 9.1	6.22	0.85	0.06	3.5– 7.9
10–15	M	277	6.90	0.96	0.06	3.9–10.2	6.26	0.98	0.06	3.2–10.1
	F	154	6.92	0.72	0.06	5.3– 8.5	6.15	0.75	0.06	4.4– 7.9
	B	431	6.91	0.88	0.04	3.9–10.2	6.22	0.91	0.04	3.2–10.1
15–20	M	154	6.88	0.82	0.06	5.2– 9.5	6.36	0.79	0.06	4.9– 8.6
	F	8	6.82	0.80	0.28	5.6– 7.9	5.68	0.54	0.19	5.0– 6.5
	B	162	6.87	0.82	0.06	5.2– 9.5	6.33	0.66	0.05	4.9– 8.6
Totals	M	547	6.86	0.94	0.04	3.9–10.2	6.23	0.93	0.04	3.2–10.1
	F	247	6.95	0.70	0.04	5.0– 8.5	6.22	0.72	0.05	4.2– 7.9
	B	794	6.89	0.88	0.03	3.9–10.2	6.23	0.87	0.03	3.2–10.1

table has been arranged according to sex and according to type of saliva (resting and activated). The age group one through five was

[3] Standard deviation $= \sqrt{\dfrac{\text{Sum of the squares of the Observations}}{\text{Number of Cases}} - (\text{Mean of Observation})^2}$.

Standard error of the mean $= \dfrac{\text{Standard deviation}}{\sqrt{\text{No. of Cases}}} = \text{S.E.}$

Standard error of the difference between two means $= \sqrt{\dfrac{(\text{S.D.}_1)^2}{N_1} + \dfrac{(\text{S.D.}_2)^2}{N_2}}$

made advisedly to include pre-school children inasmuch as their dietary and play habits were presumed to be different from those of school age children. The other age groups were made to conform with those reported in the literature for children of comparable age. Little importance can be attached to the mean values of youngest age group and of the females of age group 15–20 because of the relatively small number of cases. If we neglect these groups and use three times the

TABLE III

Frequency distribution of calcium in the resting saliva of males, females and both, for the various age groups

AGE GROUP	SEX	MGS CALCIUM							TOTAL CASES
		3.5–4.5	4.5–5.5	5.5–6.5	6.5–7.5	7.5–8.5	8.5–9.5	9.5–10.5	
yrs.									
1–6	M	0	0	3	1	0	0	0	4
	F	0	1	1	2	0	0	0	4
	B	0	1	4	3	0	0	0	8
6–10	M	3	9	25	38	31	6	0	112
	F	0	2	14	41	23	1	0	81
	B	3	11	39	79	54	7	0	193
10–15	M	1	15	69	115	64	11	2	277
	F	0	3	35	78	33	5	0	154
	B	1	18	104	193	97	16	2	431
15–20	M	0	4	36	71	38	3	2	154
	F	0	0	3	2	3	0	0	8
	B	0	4	39	73	41	3	2	162
Totals	M	4	28	133	225	133	20	4	547
	F	0	6	53	123	59	6	0	247
	B	4	34	186	348	192	26	4	794

standard error of the difference between two means as a criterion of "significance," then there are no "significant" differences between the mean values for males and females, nor between the various age groups. These findings are in agreement with those of Horton Merrack and Price (38) who reported no "significant" difference in the calcium levels of boys and girls of similar ages; and of Pattison (64) who reported no relationship between age and salivary calcium levels

in children. In studies made on adults, Becks (4) found salivary calcium to decrease between the ages of twenty and forty and increase in age groups over forty years of age; and Howe (39) reported salivary calcium to be of a higher level in individuals who have attained their growth. However, when the type of saliva is considered our data show a "significant" difference between the mean value of 6.89 mg per 100 cc. for the resting and 6.23 mg per 100 cc. for the activated

TABLE IV

Frequency distribution of calcium in the activated saliva of males, females and both, for the various age groups

AGE GROUP	SEX	MGS. CALCIUM								TOTAL CASES
		3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	
yrs.										
1–6	M	0	3	1	0	0	0	0	0	4
	F	0	1	1	2	0	0	0	0	4
	B	0	4	2	2	0	0	0	0	8
6–10	M	4	10	30	45	23	0	0	0	112
	F	0	2	15	51	13	0	0	0	81
	B	4	12	45	96	36	0	0	0	193
10–15	M	2	20	77	122	44	10	0	2	277
	F	0	7	33	81	32	1	0	0	154
	B	2	27	110	203	76	11	0	2	431
15–20	M	0	4	44	71	30	5	0	0	154
	F	0	0	6	2	0	0	0	0	8
	B	0	4	50	73	30	5	0	0	162
Totals	M	6	37	152	238	97	15	0	2	547
	F	0	10	55	136	45	1	0	0	247
	B	6	47	207	374	142	16	0	2	794

saliva. Where studies have been made on both resting and activated samples the calcium level of the resting saliva has been invariably higher than that of the activated (16), (45), (46), (56), (86).

Table III shows the frequency distribution of calcium for the resting saliva. The table has been arranged to show the frequency distribution for males, females and both in the different age groups studied. Table IV shows the same for activated saliva. The scatter around the

means for the two types of saliva is essentially the same, each being bunched rather closely around the respective mean. The distribution for the male cases extends one class farther on either side of the range than does that of the female cases. With an equal number of male and female cases this difference probably would not be apparent, i.e., the distributions are almost identical for the 154 female cases of age group 10–15 and the 154 male cases of the age group 15–20 for both resting and activated saliva.

Table V shows the frequency distribution of calcium in resting saliva for 794 cases neglecting age and sex. The first column of the table gives the classes in mgs. of calcium; the second column the number of

TABLE V

Frequency distribution of calcium of resting saliva

MGS Ca/100 CC. SALIVA	NO. CASES	PER CENT TOTAL CASES	PER CENT TOTAL CASES
3.5	4	5	5
4.5	34		
5.5	186	23	91
6.5	348	44	
7.5	192	24	
8.5	26	4	4
9.5–10.5	4		
	794	100	100

cases falling in each class; and the third and fourth columns give the percentage of the total number of cases making up each class or combination of classes. Table VI shows the same for activated saliva. Ninety-one per cent of the calcium values of the resting saliva for the 794 cases fall within the range of 5.5 to 8.5 mg calcium per 100 cc. For the activated saliva 91 per cent of the total fall within the range of 4.5 to 7.5 mg calcium per 100 cc. Thus approximately 91 per cent of the total number of cases studied, both for resting and activated saliva fall within a range of 3 mg of calcium. Theoretically ± twice the standard deviation includes 95 per cent of the total number of values. The mean ± twice the standard deviation of the resting saliva, $6.89 + (2 \times .88) = 8.65$, and $6.89 - (2 \times .88) = 5.13$, gives

TABLE VI

Frequency distribution of calcium of activated saliva

MGS Ca/100 CC. SALIVA	NO. CASES	PER CENT TOTAL CASES	PER CENT TOTAL CASES
3	6	7	7
4	47		
5	207	26	91
6	374	47	
7	142	18	
8	16	2	2
9	0		
10–11	2		
	794	100	100

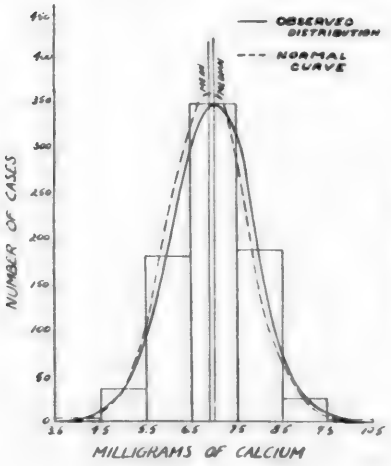

FIG. 1

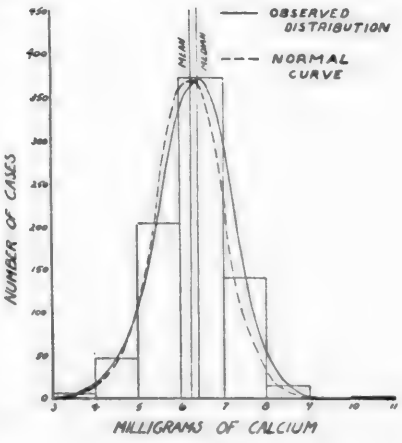

FIG. 2

FIG. 1. Histogram and fitted curve of frequency distribution of salivary calcium in resting saliva.

FIG. 2. Histogram and fitted curve of frequency distribution of salivary calcium in activated saliva.

a range of 5.1 to 8.5 or 3.5 mg; for the activated saliva, $6.23 + (2 \times .87) = 7.97$ and $6.23 - (2 \times .87) = 4.49$, the range is 4.5 to 8.0 or 3.5 mg. If in Tables V and VI we take the per cent of cases

falling in the ranges given above we have approximately 95 per cent for both resting and activated salivas.

The data in Table V is illustrated in *fig. 1* by means of a histogram with a frequency polygon and normal curve superimposed. *Fig. 2*, shows the same for the data in Table VI. The number of cases is plotted along the ordinate and milligrams of calcium along the abscissa. The mean and median are illustrated by vertical lines. The polygons for both the resting and activated salivas are of the "cocked-

TABLE VII

Studies on calcium content of resting saliva

WORKER	YEAR	AGE GROUP	NO. CASES	AVE.	RANGE
				mg. Ca./100 cc.	
Krasnow, 55	1932	19–21	25	5.3	4.0–10.3
Clark, 16	1922	Adults	3	5.6	5.3– 6.9
Roskin, 77	1927	Children	30	5.6	2.8– 7.8
White, 86	1936	9–16	25	5.8	4.9– 7.3
Jonsgar, 45	1937	?	3	5.9	3.3– 6.9
Wach, 84	1936	?	30	6.2	3.0–10.6
Leonard, 60	1928	39	1	6.5	6.4– 6.6
Present work	1938	3–17	780	6.9	3.9–10.3
Pattison, 64	1926	Children	39	7.8	?
Pattison, 64	1926	Adults	12	7.8	?
Clark, 18	1927	22–49	5	8.0	6.0–11.0
Ferris, 25	1920	?	1	8.7	8.4– 8.9
Ruthrauff, 78	1923	Adults	48	11.1	10.0–13.4
Becks, 4	1928	8–71	24	12.1	7.5–17.5
Entin, 22	1923	?	138	15.9	?
Entin	1920	?	121	16.2	5.0–33.0
Totals			1285	8.92	2.8–33.0

hat" type. The polygon for the resting saliva follows that of the normal curve, while that of the activated is skewed slightly to the right. The mean $\pm$ twice the standard deviation for both polygons includes approximately 95 per cent of the total cases expected in a normal curve.

Some of the data given in former salivary studies is summarized in Tables VII, VIII, and IX. Table VII includes studies on resting saliva, Table VIII studies on activated saliva, and IX studies in which

TABLE VIII

Studies on calcium content of activated saliva

WORKER	YEAR	AGE GROUP	NO. CASES	AVE.	RANGE
				mg. Ca./100 cc.	
Jonsgar, 45	1937	?	3	4.3	3.3– 5.9
Brown, 12	1937	Col. Students	18	4.6	3.4– 6.6
Krasnow, 55	1932	19–21	25	4.7	3.6– 7.7
White, 86	1936	9–16	25	4.8	3.9– 5.9
Hubbell, 40	1932	7–16	102	5.2	?
Hubbell, 41	1933	9–16	32	5.3	4.1– 6.6
Karshan, 46	1931	19–25	44	5.5	3.5– 9.2
Koehne, 53	1934	6–13	22	5.5	3.9– 7.7
Karshan, 48	1936	13–42	105	5.6	?
Present work	1938	3–17	780	6.2	3.1– 9.0
Hatton, 32	1937	?	20	6.6	4.0– 8.0
Fosdick, 27	1937	?	5	7.0	6.0– 8.0
Rose, 76	1905	12–14	219	7.6	5.3–11.9
Rickert, 75	1924	Adults	31	7.6	3.5– 8.6
Horton, 38	1929	3–12	741	7.8	2.0–13.0
Leonard, 60	1928	39	1	8.0	6.0–11.8
Ruthrauff, 78	1923	Adults	48	12.9	10.0–17.4
Totals			2221	6.92	2.0–17.4

TABLE IX

Unclassified studies of calcium content of saliva

WORKER	YEAR	AGE GROUP	NO. CASES	AVE.	RANGE
Frerichs, 28	?	?	?	50.71 parts CaO/1000	
Hammerbacher, 29	1881	?	?	5.01 parts CaO/100	
Bunting, 13	1915	Students	19	?	4.3– 12.0
Bunting, 14	1917	Students	30	?	4.3– 10.0
Broderick, 9	1920	?	?	CaO crystals	
Janssen, 44	1926	?	?	Ca/hr 17.2–19.4	
Clark, 17	1927	?	5	?	4.0– 6.0
Knoche, 54	1927	?	76	?	12.2– 17.8
Fetterly, 26	1931	Students	52	42	?
Hawkins, 36	1931	?	140	?	15.0–145.0
Price, 69	1932	?	25	6.0	4.5– 10.0
Hansen, 31	1933	?	?	?	?
Mathis, 61	1934	?	6	?	4.5– 6.0

the type of saliva was not reported. In some instances in which the calcium was determined as the oxide or the carbonate, the values have been converted to mg of calcium per 100 cc. of saliva. For the sixteen

studies listed, on the resting saliva the means varied from 5.3 to 16.2 mg per 100 cc. with an average of 8.92 mg per 100 cc. The range for the individual determination was 2.8 to 33.0 mg per 100 cc. for a total of 1299 cases. In the seventeen activated salivary studies listed the means varied from 4.3 to 12.9 mg per 100 cc., with an average of 6.92 mg per 100 cc. These studies included a total of 2235 cases with a range of from 2.0 to 17.4 mg per 100 cc. for individual cases.

As pointed out before these large differences in the results reported by former workers are probably due to differences in precision of methods used, and failure to give preliminary treatment to the saliva.

SUMMARY AND CONCLUSIONS

1. The need for the establishment of standard values for salivary calcium, using an acceptable method for its determination is discussed. 2. Former salivary calcium studies are cited and listed in table form. 3. The Clark-Collip modification of the Kramer-Tisdall method for serum calcium, modified for use on saliva is outlined. 4. Calcium determinations were made on the resting and activated salivas of 794 apparently healthy school children, (547 males and 247 female cases), ranging in age from 3 to 18 years, with an average age of 11.6 years. 5. No "significant" differences exist between the mean calcium values for males and females in either the resting or activated saliva. 6. There are no "significant" differences between the means of the various age groups studied (1–6, 6–10, 10–15, and 15–20) for either type of saliva. 7. Disregarding sex and age, the calcium content of the resting saliva of the 794 cases studied have a mean calcium value, standard deviation, standard error of the mean, and ranges of individual determinations of respectively 6.88, ± 0.88, ± 0.03, and 3.9 to 10.2 mg calcium per 100 cc.; for the activated saliva 6.23, ± 0.87, ± 0.03 and 3.2 to 10.0 mg calcium per 100 cc. saliva. 8. The difference between the means of the resting and activated saliva of .66 mg of calcium per 100 cc. (6.89–6.23) is "significant." The criterion of "significance" used was three times the standard error of the difference between two means. 9. The distribution of the calcium values around the mean is of a relatively symmetrical nature. The three central classes, 5.5, 6.5 and 7.5 for the resting saliva, and 5, 6 and 7 for the activated saliva, include approximately 91 per cent of the total number of cases studied. Using ± twice the standard deviation

around the mean, 95 per cent of the total number of cases are included. This is the percentage of the total cases expected theoretically, assuming that the curve is normal. 10. It may then be concluded, that a standard range for salivary calcium for children 6 to 18 years of age would be: resting saliva 5.1 to 8.6 mg calcium per 100 cc.; activated saliva, 4.5 to 8.0 mg of calcium per 100 cc. saliva. Theoretically, these values should include approximately 95 per cent of all determinations made on a similar group. 11. So far as any individual determination is concerned, the use of salivary calcium as a diagnostic tool would have a rather limited usefulness due to the wide range in standard values. However, mean calcium values, derived from the study of a fairly large number of cases, would be of value in correlation studies.

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THE EFFECT OF SALIVA ON LACTOBACILLUS ACIDOPHILUS[1, 2]

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The factors which determine the characteristics of the microbial flora of the oral cavity have been but little investigated and are only poorly understood. The mechanical removal of organisms from the mouth (1, 9) does not account adequately for the existence of a distinctive oral flora. It is possible that saliva may exert a selective action on bacterial growth as indicated by preliminary experiments (4). In view of the prominent rôle which has been assigned by certain workers (2, 3, 5) to *Lactobacillus acidophilus* as the causative agent of dental caries, the effect of saliva on this organism was investigated.

MATERIALS AND METHODS

Lactobacilli. Three strains of *Lactobacillus acidophilus*, isolated from cases of dental caries, were used. These strains were "Type I" and "Type II," isolated by Hadley (7), and the "Type I" isolated by McIntosh, James and Lazarus-Barlow (8). Because results were easier to read with the Hadley-Type II strain, this organism was generally used. Since the other strains which were also used from time to time gave consistently positive results it is felt that the findings with the test strain may be accepted as representative of the oral lactobacilli.

Culture Media. Douglas' broth containing 5 per cent of glucose was used for maintaining the stock cultures. Tests for "salivary effect" were made in plates of Kulp's tomato juice agar, seeded with 2.5 per cent of a 40-hour broth culture. The clearest results were obtained when the agar was prepared with tomato juice which had been passed through filter paper. The agar in the Petri plates did not exceed 2.5 mm. in thickness. Wells, 11 mm. in diameter, were cut in these plates with sterile test tubes and

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were filled with saliva or control fluids, such as 0.85 per cent sodium chloride solution or distilled water. After 48 hours incubation at 37°C, the plates were examined. Where inhibition of growth had occurred, a clear zone containing no colonies surrounded the well (*fig. 1*). Otherwise, colonies developed up to the edge of the well. To provide an index of the action of the saliva, the widths of the colony-free zones surrounding the wells were measured in mm.

Saliva. The saliva used in these tests was collected in sterile centrifuge tubes without employing any method of activation. Samples of 50 cc. were contributed by certain individuals for some of the special tests, but routinely amounts of 2 cc. sufficed. In all,

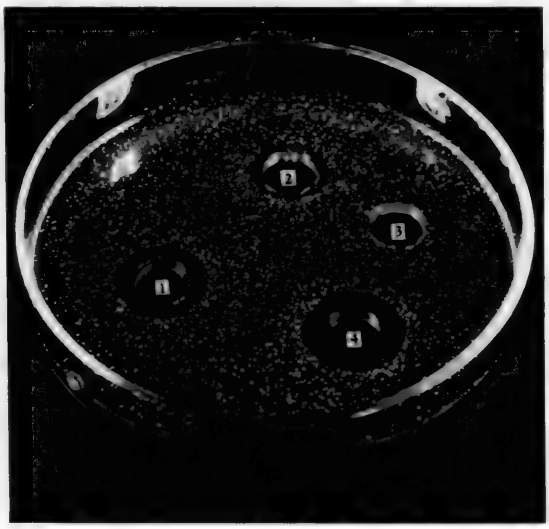

FIG. 1. Poured tomato juice agar culture of *Lactobacillus acidophilus* showing colony-free zones about wells 1 and 4 which contained salivas from different individuals. A narrower zone is seen about well 2 which contained first active portion of saliva to pass Berkfeld W filter. No zone is apparent about well 3 which was filled with 0.85 per cent NaCl solution.

saliva was collected from 204 individuals. Of these, 77 were healthy adults from 20 to 60 years of age, 77 were children between the ages of 4 and 16 years (examined at the Rochester Dental Dispensary and the Hillside Home for Children), 25 were diabetic patients under hospital treatment, and 25 were women between the fourth and ninth month of pregnancy.

FINDINGS

Occurrence of Lactobacillus-Inhibitory Agent. In 260 tests made with the saliva obtained from 204 individuals some growth-inhibition

of lactobacilli occurred in 250. No colony-free zones appeared around wells containing control fluids. In Table I the widths of the zones in the groups of adults, children, diabetic patients and pregnant women are compared. No significant differences in the inhibitory potency of saliva from the various groups was apparent.

To determine whether any correlation existed between the growth inhibitory potency of the patients' salivas and the patients' susceptibility to dental caries, a comparison was made of the zone-width given by the salivas of the 77 children and the amount of dental caries present. The number of carious teeth was determined by means of examination with mouth mirror and dental explorer. Although the amount of caries varied from 1 small filling to 18 open

TABLE I

Degrees of growth-inhibition of Lactobacillus acidophilus observed in a study of salivas from several groups of individuals

GROUP	NO. OF INDIVIDUALS	NO. OF TESTS	NO. SHOWING NO INHIBITION	NO. SHOWING INHIBITION. WIDTHS OF COLONY-FREE ZONES			
				1 mm.	2 mm.	3 mm.	4 mm.
Healthy Adults	77	133	2	14	13	25	79
Healthy Children	77	77	8	7	19	21	22
Diabetic Patients	25	25			8	6	11
Pregnant Women	25	25			9	6	10

cavities and 5 fillings, no correlation could be established between the extent of caries and the degree of inhibition demonstrated in the plates.

Mode of Action of Inhibitory Agent. To find out whether the colony-free zones appearing about saliva-containing wells were manifestations of a bactericidal or merely a bacteriostatic action of the saliva, the colony-free zones were tested for the presence of viable organisms. On 5 tomato juice agar plates, inoculated with agar removed on a sterile platinum loop from colony-free zones, colonies failed to develop. Five plates inoculated in the same way from test plates stored in the refrigerator for 24 hours gave copious growth in each instance.

To test for possible lytic effects of the saliva, suspensions of lactobacilli in various dilutions of saliva were incubated at 37°C for 42

hours. For this purpose 0.4 cc. amounts of a bacterial suspension containing approximately 2,500 million organisms per cc. were used in saline dilutions of saliva at titers of from 1:2 to 1:1280. No lysis appeared in any tube. Subcultures made after 18 hours of incubation showed viable organisms in all of the tubes, but after 42 hours viable organisms could be recovered only from tubes in which the dilution was greater than 1:80.

Nature of Zone-Producing Agent. A few tests were made to determine whether the active principle was an actual component of the salivary secretion or an incidental product occurring in saliva as the result of bacterial metabolism or cellular degeneration. A few examinations were also made to find out in what ways the growth-inhibitory principle resembled other biological agents.

In all instances a comparison of the "zoning-effect" of 6 samples of whole saliva, of the sediments produced by centrifuging at 2,200 r.p.m., and of the supernates showed that the supernates gave zones of the same width as those produced by whole salivas. It was obvious, therefore, that the anti-bacterial effect did not depend on the presence of salivary epithelial cells and corpuscles.

Filtrations of centrifuged saliva through Berkfeld W filters showed that the first 10 cc. of the filtrate were ineffective against lactobacilli, but that subsequent portions of the filtrate produced the usual colony-free zones. This observation demonstrated that "zoning" occurred after the bacteria had been removed. It also showed that the agent was adsorbed on the filter, and was presumably negatively charged.

To test further the possibility that products of bacterial growth produced an inhibition of the growth of lactobacilli, another series of tests was carried out. Wells, in seeded plates, were filled with (**1**) Douglas' broth heavily inoculated with salivary centrifugate, (**2**) Douglas' broth heavily inoculated with a 24-hour culture of oral bacteria, (**3**) a Berkfeld W filtrate of a 24-hour Douglas' broth culture of oral bacteria, and (**4**) a Berkfeld W filtrate of a 24-hour culture of salivary centrifugate in equal parts of Douglas' broth and ineffective salivary filtrate. The absence of zones of inhibition about any of the wells suggested that the agent was an inherent part of the salivary secretion.

The Effect of Heat. Four 3 cc. specimens of filtered saliva, not

including the first 10 cc. collected, were heated in 15 mm. Pyrex test tubes in water baths at temperatures of 55°, 60°, 65°, 70° and 75°C. After 5, 10, 15, 20, 25 and 30 minutes at these temperatures, samples of saliva were tested for anti-bacterial properties. It was found that the agent withstood a temperature of 65°C for 30 minutes. After 5 minutes at 70°C the colony-free zones were narrower. Three of 4 salivas were completely inactivated after 25 minutes at this temperature. Five minutes at 75°C completely inactivated all the samples of saliva tested.

The Effect of Ultraviolet Light. Three 1 cc. specimens of centrifuged saliva were placed in 90 mm. Petri dishes and exposed to ultraviolet radiation from an Alpine Sun Lamp at a distance of 75 mm. After exposures of 1, 2, 3 and 4 minutes, the zoning effect of the irradiated salivas were tested. Exposures of 3 minutes inactivated the anti-bacterial agent and the shorter exposures produced proportionate decreases in the widths of the colony-free zones.

The Effect of Freezing and Thawing. Four 1 cc. samples of the effective fractions of Berkfeld filtrates of saliva were subjected to 8 alternate freezings and thawings. Freezing was accomplished in a carbon dioxide-ethyl alcohol mixture at about −70°C; thawing took place in a water bath at 45°C. Each combined freezing and thawing required about 1½ minutes. The growth inhibitory potency of the salivas was tested after each thawing. It was found that 2 freezings decreased the width of the zones formed in the test plates, while 4 freezings completely destroyed the zoning action.

DISCUSSION

The experiments described in the present report demonstrate that there is present in saliva an agent which is capable, under the conditions of the experiment, of preventing the growth of, and even of killing, *Lactobacillus acidophilus*. Under other conditions, for example in suspensions of lactobacilli in saliva, the effect is comparatively weak. The reasons for this difference in activity are not clear.

So far, we are able to offer but little evidence regarding the nature of the anti-bacterial agent. The zoning brought about after the bacteria have been removed from the saliva and our failure to produce colony-free zones with filtrates from cultures of oral bacteria

suggest that the active agent is probably a component of the salivary secretion. In the adsorption of the agent during filtration and in the inactivation by heat, there is a resemblance to Fleming's lysozyme (6). The destruction of the agents by ultraviolet light and by repeated freezings and thawings offers support to the possibility that the agent is a protein. It is clear, however, that these preliminary studies justify no final conclusions as to the nature of the agent.

In view of the prominence which has been given to claims that *Lactobacillus acidophilus* is the cause of dental caries, it is interesting to find that saliva is capable of preventing the growth of this organism *in vitro*. Its rôle in the mouth in this connection is a matter for conjecture only, but it can be ignored in considerations of the etiology of caries only when it has been shown that the effects demonstrated *in vitro* do not operate at all in the mouth.

SUMMARY

Zones free of bacterial growth were produced in agar plates of *Lactobacillus acidophilus* about wells containing 250 of 260 salivas tested. These zones were shown to be manifestations of a bactericidal effect of the saliva. The active principle appeared to be a component of the salivary secretion. It was destroyed in 5 minutes at 75°C. Ultraviolet radiation and repeated freezings and thawings also inactivated it. The inhibitory potency of the saliva could not be correlated with the state of oral health in the individual from whom it was secured.

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DESTRUCTION OF TOOTH ENAMEL BY ACIDIFIED CANDIES[1]

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Miller and Neuwirth (1) have recently reported a series of cases of acute dental caries in which the sudden incidence of the condition was attributed to excessive indulgence in hard candies with resultant acid formation through bacterial decomposition. This explanation is in accord with current theories (2) as to the mechanism by which carbohydrates participate in dental decay. It seemed to the writers that possibly another factor, apparently little considered, may be of importance, namely, the presence of citric acid in many hard candies. According to Neuwirth[2] the candies concerned in their observations were not of the acidified variety. Citric acid is one of the stronger organic acids and forms soluble, poorly ionized complexes with calcium. It would appear therefore that citric acid contained in candies may be of far more importance in tooth destruction than the acid produced from the sugar by bacterial decomposition in the mouth. Experimental evidence is here presented which seems to give support to this idea.

The results of our in vitro experiments suggest that the action of citric acid solutions upon tooth enamel proceeds in two ways. There is the action due to the acidity of the solution leading to the formation of soluble calcium citrate complexes. There is also a destruction of the enamel due to the citrate ion of the solution replacing the phosphate ion in the surface of the enamel, thereby changing its chemical composition and crystal structure.

EXPERIMENTAL

Freshly extracted teeth from the Outpatient Dental Clinic of the University of Oregon Medical School were used in the experiments designed to test the destructive action of

[1] Presented in part at the meeting of the American Society of Biological Chemists, Baltimore, March 30–April 2, 1938.

[2] Personal communication.

solutions of various acidified candies upon tooth enamel. The teeth were mounted singly in rubber stoppers by imbedding the roots in beeswax followed by a coat of acid proof paint. Only sound enamel structure was exposed. The stoppers with teeth mounted were then placed firmly in the mouths of two-ounce bottles containing an accurately

TABLE I

Showing the dissolving action of acidified candies upon tooth enamel

TOOTH NUMBER	SOLUTION USED	pH		HRS. RUN	TOTAL Ca DISSOLVED; CALCULATED AS MG. OF ENAMEL. (3)*
		Start	End		
	Series A, water solutions				
1	10% Orange Drops, Brand "A"	2.8	3.5	25.5	18.1
2	20% Orange Drops, Brand "A"	2.6	3.3	25.5	26.5
4	40% Orange Drops, Brand "A"	2.6	2.9	24	26.2
6	60% Lemon Drops, Brand "A"	2.5	2.7	24	21.4
13	20% Lime Drops, Brand "A"	2.8	3.6	24	25.4
	Series B, 20% in saliva				
7	Lime Drops, Brand "A"	5.0	5.0	24	1.0
9	Wild Cherry, Brand "B"	5.2	5.2	24	1.30
10	Orange Drops, Brand "B"	5.0	5.2	24	1.3
11	Pineapple, Brand "B"	5.4	5.6	24	0.7
12	Lemon Drops, Brand "B"	5.0	5.3	24	1.2
	Series C, 40% in saliva				
14a	Lime Drops, Brand "A"	3.5	3.5	22.5	10.0
8a	Lemon Drops, Brand "A"	3.4	3.6	22.5	10.4
3a	Lemon Drops, Brand "B"	3.6	3.7	22.5	5.7
15	Orange Drops, Brand "B"	3.7		22.5	3.1
19	Lime Drops, Brand "A"	3.5	3.9	24	7.2
20	Lemon Drops, Brand "B"	3.7	3.9	24	7.1
18	0.1 N Lactic Acid and Sucrose	3.2	3.5	24	28.4

* The factor determined from the figures of Bowes and Murray (3) is 2.70. These figures are carefully calculated and take into account the calcium in solution before the teeth were exposed and also the amount lost in withdrawals for determinations done in most instances during the course of the experiment; i.e., in Series B, at 1 and 6 hours as well as at the end of 24 hours.

measured quantity (30–50 cc.) of the solution to be tested and shaken at room temperature for definite periods of time in a mechanical shaker. In this way the tooth enamel was continuously in contact with the solution. The difference in the calcium content of the solutions before and after agitation with the teeth gave the net calcium dissolved from the enamel. The enamel equivalent of the calcium dissolved was calculated by multiplying

the calcium by 2.7, the factor of Bowes and Murray (3). pH determinations were made on the solutions, before and after agitation with the teeth, by the quinhydrone electrode. Solutions were preserved during the experiments with a drop or two of 10 per cent thymol in chloroform. Saliva for the experiments was collected while chewing paraffin and then filtered. Table I summarizes the results obtained.

It will be noted that strong solutions of acidified candies in water are really acid, showing pH values of 2.5–2.8 (40 per cent lime, lemon, and cherry candies in water were 0.079; 0.054 and 0.071 N acid by titration). Such solutions have a pronounced solvent action upon enamel. Twenty per cent solutions of the various acidified candies in normal saliva showed much lower acidities, the pH values ranging from 5.0–5.4. Such solutions definitely dissolve enamel though not to any great extent. When the concentration of the candies in the saliva was raised to forty per cent, however, the pH dropped to 3.4–3.7 (0.1 N lactic acid in 40 per cent sucrose gave a pH of 3.2) and the destructive action upon the enamel became marked. The time of action of the solutions on the teeth given in the table was 22–25.5 hours. Observations were made on other teeth at 1, 6, and 24 hours. The 20 per cent solutions of acidified candies in saliva showed no solution of enamel in 6 hours but a definite amount in 24 hours. On the other hand the 40 per cent solutions showed definite action on the enamel in 6 hours.

In general, as expected, the destruction of enamel was greater the lower the pH of the solution. Unavoidable variation in the teeth used in testing the different solutions undoubtedly had some effect and caused minor variations. Teeth which have been air dried for 10 months after extraction were found more resistant to attack than the freshly extracted teeth used in the above experiments.

Strong solutions of sucrose, levulose and non-acidified candy did not dissolve tooth enamel.

The amount of calcium dissolved from the enamel surface did not represent all of the tooth destruction. A chalky insoluble deposit remained on the surface of all teeth which had shown marked solution by the acid candies. Some of this material was collected and found to have a Ca/P ratio of 2.3 as contrasted with a value of 1.67 for tooth enamel (3). This observation suggested that the citrate ion from the solution had replaced the phosphate ion in the enamel.

Such an assumption was made more probable by observations upon "synthetic" tricalcium phosphate, prepared according to the directions of Mellor (4). This material is probably of the apatite type of salt, having calcium hydroxide or carbonate combined with the phosphate (5). Our material evolved carbon dioxide when treated with acid and thus contained some carbonate. The Ca/P ratio of the salt was 1.69, very close to the values of 1.66 for apatite and 1.67 for the mineral of tooth enamel.

0.5 gm. samples of the synthetic calcium phosphate were placed in bottles with 50 ml. portions of 0.1 M citrate buffers (pH 2.91–5.05) and mechanically shaken for nine days in an incubator room at 38°C. At the end of this time Ca and P determinations were run

TABLE II

Effect of 0.1 M citrate buffers upon synthetic tricalcium phosphate at different pH values. Equilibrated for 9 days at 38°C.

EXP. NO.	pH CHANGE	Ca DIS. M.MOLS.	P DIS. M.MOLS	Ca/P LIQUID	Ca/P SOLID
1	2.91–3.39	1.65	2.6	0.63	7.9
2	3.15–3.72	1.15	2.5	0.46	6.4
3	3.56–3.98	0.85	2.5	0.33	5.8
4	3.88–4.23	0.80	2.5	0.31	5.4
5	4.21–4.73				4.5
6	4.45–5.05				4.4
7	4.75–5.20				1.66
8	5.05–5.40				1.65

on both the undissolved solid and the solutions and Ca/P ratios calculated. Determinations of the pH of the solutions before and after each run were made. At a final pH value of 4.73 and above the solutions became opalescent due to colloidal particles and Ca and P estimations were omitted. Table II summarizes the data obtained.

The Ca/P ratios in the solutions after action on the solid progressively declined with rising pH and only in experiment 1 was the ratio high enough to represent a phosphate of calcium, ($Ca(H_2PO_4)_2$). In other words, much more phosphate than calcium ion dissolved. This fact is brought out more strikingly by the Ca/P ratios of the undissolved salt. In experiment 1 the Ca/P ratio of the remaining salt was found to be 7.9 and not until the final pH rose to 5.20 in experiment 7 did the ratio approach that of the untreated salt (1.69).

This shows that phosphate ion dissolved from the solid phase without equivalent calcium ion solution, which necessarily leads to the conclusion that citrate ion replaced phosphate ion in the solid phase. The sharp break in the solid Ca/P ratio between pH values of 5.05 and 5.20 is of interest. Apparently the citrate-phosphate ionic exchange without solution of the solid phase does not occur above pH 5.20.

DISCUSSION

When an individual places a piece of ordinary acidified candy in his mouth and allows it to dissolve slowly against his teeth the concentration of the solution at the surface of the candy (and in contact with the tooth enamel) will be very high with a probable pH of 3–4. If the individual repeats the process often throughout the day and makes a daily habit of it our experimental data indicate that serious decalcification is likely to result. The occasional use of such candies undoubtedly has little effect. Due to the fact that the temperature in the mouth is considerably higher than in our experiments (20–25°C.) the rate of solution would be increased. However, the buffering action of the saliva in the mouth, where it is being continually secreted, is undoubtedly more efficient against acidified candies than in the experiments reported. The importance of the buffer action of saliva in overcoming the acidity of the candy should be emphasized. Normal saliva protected rather well against the dissolving action of 20 per cent acidified candy but poorly in the more concentrated solutions. The destructive action on the teeth of different individuals should, accordingly, vary with the buffer capacity of the saliva secreted. Another fact should be pointed out. The amount of enamel dissolved does not represent the total destruction. The teeth treated in our experiments under conditions that led to marked solution of tooth mineral showed a chalky insoluble layer on the surface which could be easily scraped off. The Ca/P ratio in this material was high due to the exchange of citrate ion of the solution for the phosphate ion of the enamel. The writers are of the opinion that this phenomenon of ionic exchange between tooth enamel and acidic groups of various

foods may be an important factor in the general process of tooth destruction throughout the years of tooth life.

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VASCULARITY OF THE HUMAN ENAMEL ORGAN[1]

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Despite several studies upon the question of the vascularity of the enamel organ of mammalian teeth, recent textbooks on dental histology, (5, 12, 18, 19, 21, 22, 25) except for the text by Broomel and Fischelis (9), either make no mention of this subject or specifically deny that blood vessels penetrate the enamel organ. The attention of many students of dental embryology and histology has been directed to the cytomorphosis of the enamel organ and the manner in which the hard structures of the teeth are formed, but the mechanism of nutrition by which this function is achieved within the enamel organ has received much less attention.

The literature contains a number of references to the question of the vascularity of the enamel organ in various mammalian species by earlier workers in the field. These include Paul (1), Williams (2), Lepkowski (3), Hopewell-Smith and Tims (4, 5), Bolk (6, 7), Carter (8), Skillen (10), Mummery (11, 12), Sprawson (13), and Jordan (14, 15). Although these writers have recorded their findings on this question, considerable confusion remains, because in very few instances has this problem been the principal object of the investigation, or have the species and the age of the subject and the position of the teeth described, been mentioned.

An important contribution was made by Addison and Appleton (16), who described in detail the vascular penetration of the stellate reticulum of the rat molar as early as the twenty-first fetal day. Kingery (17) confirmed this work and reported similar findings in the molars of the pig and of man subsequent to the fifth month of gesta-

[1] Presented at the 16th General Meeting of The International Association for Dental Research, Minneapolis, Minn., March 12–13, 1938.

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tion. Boyle (20) reported vascular penetration in a patient with congenital lues. More recent reports by Santoné (23) and Lehner and Plenk (24) reaffirm the presence of blood vessels within mammalian tooth germs. The paper by Lehner and Plenk gives an extensive bibliography of the subject.

PROCEDURE

This report is based upon a series of 13 examples selected as representative of different ages and pathological conditions from patients coming to postmortem examination at The Children's and Infants' Hospitals of Boston. The material in each instance consists of a portion of the mandible removed at necropsy and includes one or both deciduous molar tooth germs, that of the permanent first molar, and in one instance germs of two deciduous anterior teeth. The specimens were radiographed following removal, fixed in 10 per cent formalin, Regaud's, or Bouin's solution, decalcified in 5 per cent aqueous nitric acid, imbedded in celloidin, and the sections stained with Mallory's aniline blue connective tissue stain or alum hematoxylin and eosin. Mallory's stain is particularly suited for this work because it stains the erythrocytes in the capillaries a bright orange or red in contrast to the epithelial tissues. Table I summarizes the cases included in this series. They range in age from a premature infant born during the sixth fetal month and living 6 days, to a full-term infant 14 months of age. A wide selection of pathological conditions is represented in order to meet the possible criticism that this vascularity of the enamel organ is a result of any particular disease. The findings in patients 1, 2, and 10 are particularly significant inasmuch as neither gross nor microscopic examination indicated any significant amount of infection.

FINDINGS

The presence of blood vessels within the enamel organ has been observed so frequently in the human material in this laboratory that we believe the statement is justified that vascular penetration of the enamel organ occurs in all posterior teeth in man after the deposition of enamel and dentin has begun.

Toward the close of the period of cell differentiation the outer enamel epithelium proliferates in an irregular manner so as to form papillae. Coincidentally the blood vessels of the connective tissue capsule which invests the enamel organ extend down into invaginations between the papillae and form a rich capillary plexus in close relation to the papillary layer (*fig. 1*). The first actual invasion of the enamel organ occurs at the bases of these invaginations between the papillae of the outer enamel epithelium. The vessels pass through these openings, first described by Mummery (11), into the stellate reticulum. Associated with the penetration into the reticulum is the development of perivascular spaces (*fig. 2*). These

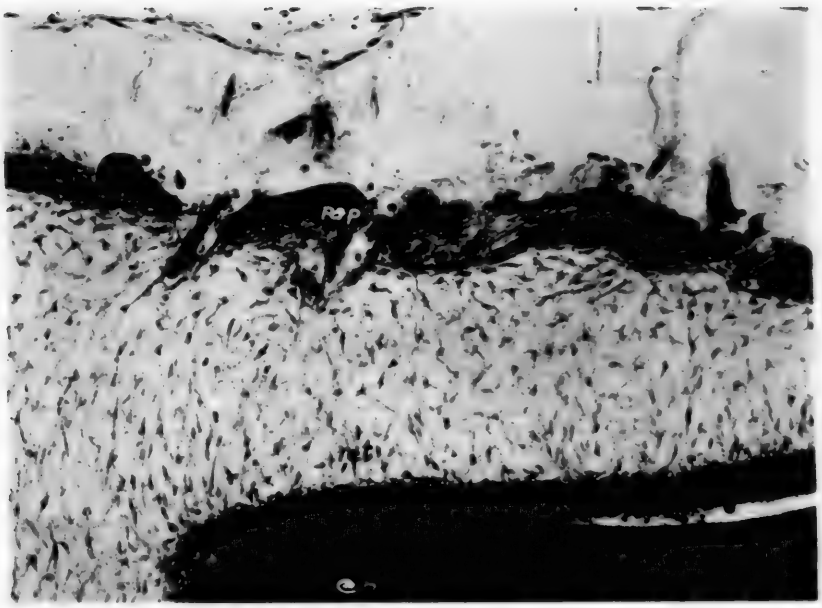

FIG. 1. Case 2, anterior cusp of deciduous second molar. Aniline Blue. 160 ×; en. enamel; ret, stellate reticulum; pap, papillae of outer enamel epithelium; cap, capillary.

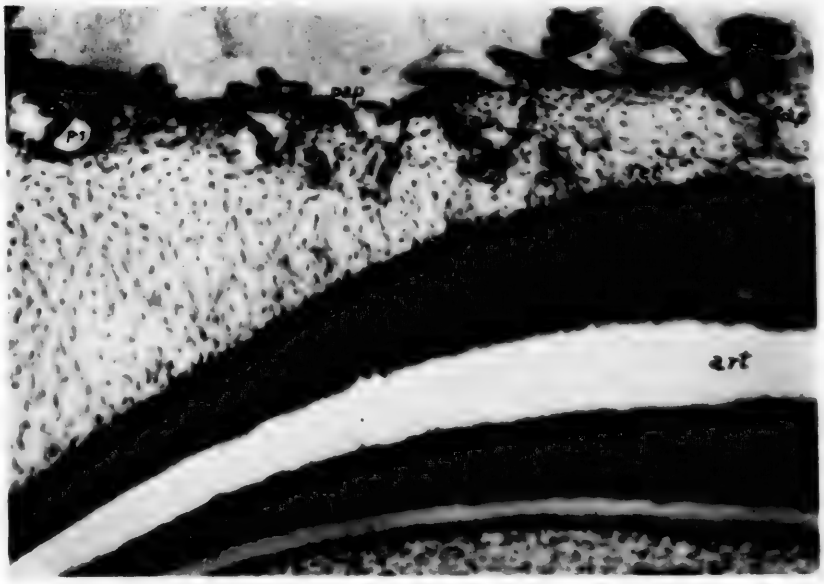

FIG. 2. Case 10, posterior cusp of permanent first molar. H and E. 118 ×; art. artefact; ps, perivascular space.

quickly assume the appearance of an almost continuous row of round or oval spaces marking the border between the outer epithelium and the reticulum. Small vessels may be observed lying within one or more of these spaces in any selected section (*fig. 3*). The earliest penetration seems to occur near the tips of the developing cusps on the side facing the central fossa. The reticulum usually has already been reduced in width in this area by the time penetration occurs. However, concurrent with the marked reduction of the reticulum over the cusps or its complete disappearance, these capillaries

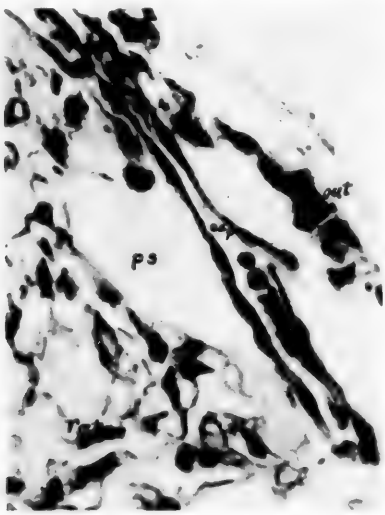

FIG. 3. Case 7, deciduous second molar H and E. 800 ×; out, outer enamel epithelium.

piercing the outer epithelium and perivascular spaces appear over the central fossa, often in an area where the reticulum retains its maximum width.

While the reticulum is undergoing involution, the blood vessels become more numerous within the enamel organ, but do not penetrate very far beyond its outer border. By the time the reticulum has been reduced to 0.1 to 0.2 mm. in width the capillaries lie much nearer to the stratum intermedium, although still no deeper with respect to the outer border of the reticulum. Meanwhile the outer

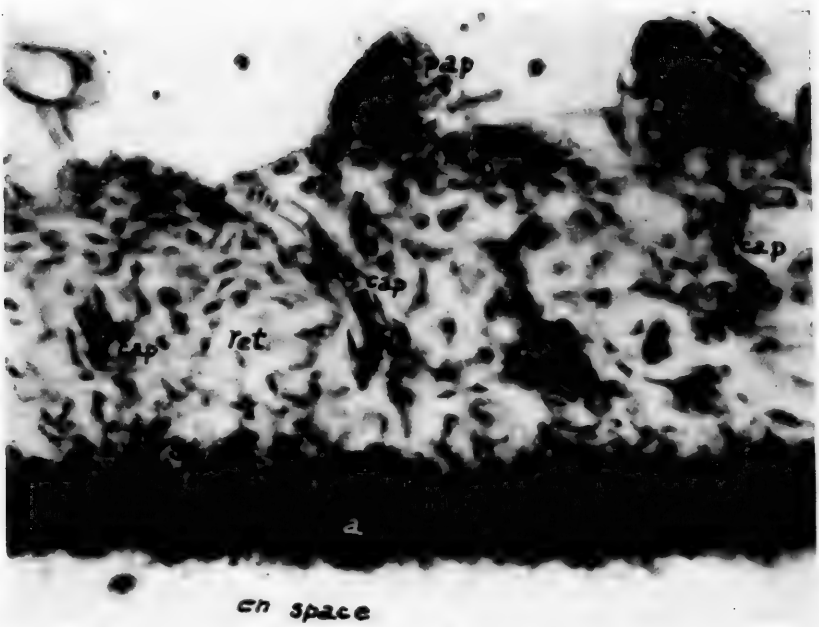

FIG. 4. Case 5. Deciduous second molar. H and E. 475 ×; st. stratum intermedium; a, ameloblasts.

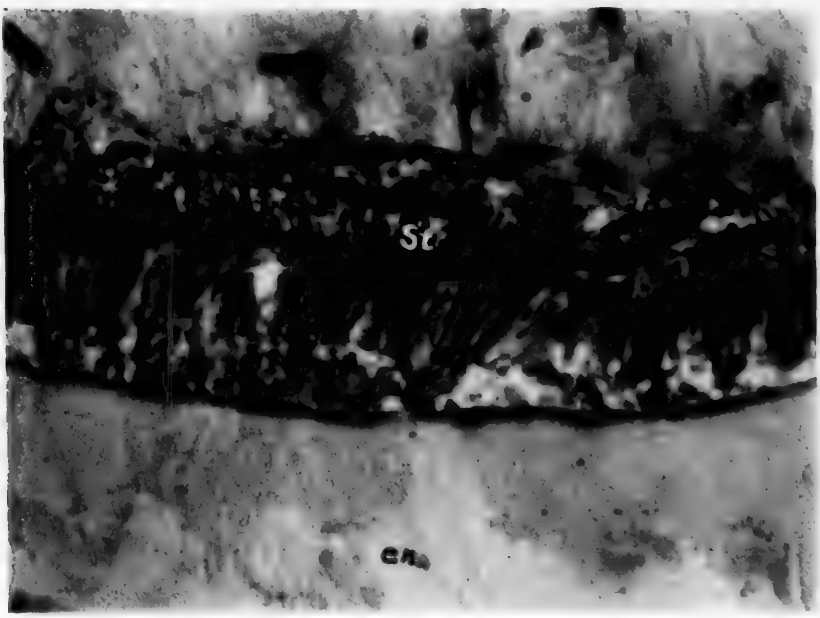

FIG. 5. Case 11. Deciduous first molar; Aniline Blue. 231 ×

epithelium has lost its continuity in certain areas over the occlusal surface. The papillae become separated as the capillaries pass through this layer in greater numbers (*fig. 4*).

Shortly before the reticulum disappears, the outer epithelium once more becomes continuous except for places through which the larger vessels penetrate, and the papillae become less prominent. Concomitantly the vessels are seen entering the stratum intermedium.

FIG. 6. Case 4, deciduous canine: Aniline Blue. 156 ×

During this late stage of enamel organ involution, large capillaries are found within the stratum intermedium following a course which is in general parallel to the surface. In several instances where they penetrate deeper toward the ameloblastic layer, the ameloblasts lying nearest to the blood vessel exhibit signs of rapid involution. The nuclei become pyknotic and acidophilic in their staining reaction, while the outline of the cells resembles that of squamous epithelium (*fig. 5*).

In a given specimen several of these stages in the vascular penetra-

TABLE I

CASE	AGE	DIAGNOSES	TEETH EXAMINED	FINDINGS
1	6 days (Length 33.5 cm.)	Prematurity, Bronchopneumonia	IV, V	Prominent papillary layer of outer epithelium in intimate contact with capsular capillary plexus. Vascularization of reticulum near enamel formation and where reticulum is reduced. Large perivascular spaces with early vascular invasion of reticulum. Numerous vessels near stratum intermedium where reticulum is markedly reduced in width, notably on anterior aspect of IV.
2	0 days (Length 40 cm.)	Prematurity	IV, V	Retarded dental development—wide stellate reticulum over central groove. Early formation of papillary layer adjacent to forming enamel in V. Capillaries invading outer epithelium but no penetration of reticulum. (*Fig. 1*)
3	15 days	Bronchopneumonia, Jaundice	V, 6	No calcification apparent yet by X-ray in 6. Well developed papillary layer in V, adjacent to forming enamel Wide variation in width of reticulum between cuspal areas and over occlusal fossae. Large perivascular spaces in outer part of reticulum. Penetration of vessels into reticulum through outer epithelium without perivascular spaces.
4	16 days	Erysipelas, Streptococcus septicemia	II, III, IV, V, 6	Papillae of outer epithelium in II are nearly separate and are surrounded by rich capillary plexus. Vessels penetrate into narrow reticulum over lingual fossa and on labial surface near cervical border. Vessels penetrate to ½ width of reticulum on labial surface of III. In IV and V vessels are present in outer part of reticulum. In 6-perivascular spaces are present beneath outer epithelium with early vascular penetration. (*Fig. 6*)

TABLE I—*Continued*

CASE	AGE	DIAGNOSES	TEETH EXAMINED	FINDINGS
5	6 weeks	Congenital Lues	IV, V	In IV a "united epithelium" covers cusps. Reticulum is reduced in width over fossa and sides of cusps. Outer epithelium is very irregular and almost pedunculated, apparently about to become discontinuous. Rich plexus in outer part of reticulum. Occasional vessels penetrate deeply toward stratum intermedium where outer epithelium has broken up. (*Fig. 4*)
6	7 weeks	Epidermolysis bullosa, Septicemia	IV, V, 6	In IV the capsular plexus sends vessels into the narrowed reticulum through a fenestrated outer epithelium. Less vascular penetration is seen in V. The reticulum is wider, while the outer epithelium is still continuous containing different sized papillae. Occlusal surface of 6 has early vascular penetration of the outer epithelium and reticulum. Perivascular spaces in the reticulum over the fossa contain capillaries.
7	2½ mos.	Acute Nutritional Disturbance, Mongolism, Rickets	III, IV, V	V has narrowing of reticulum above cusps. Many capillaries are present within perivascular spaces beneath outer epithelium on sides of cusps. (*Fig. 3*)
8	3 mos.	Congenital Lues	V, 6	Rich vascular plexus in greatly reduced reticulum in V. Opposite narrowest part of reticulum vessels enter stratum intermedium. In 6 many perivascular spaces are beneath outer epithelium opposite wider part of reticulum.
9	4 mos.	Whooping Cough, Bronchopneumonia	V, 6	Greatly reduced reticulum in V contains vessels running parallel to surface. Stratum intermedium appears wider than in Case 8. There are large perivascular spaces. In 6 reticulum is regular in width except over fossa. Numerous perivascular spaces contain capillaries.

TABLE I—*Concluded*

CASE	AGE	DIAGNOSES	TEETH EXAMINED	FINDINGS
10	5 mos.	Hydrocephalus	V, 6	V is covered by "united epithelium" except for small remnant of reticulum at cervical border. Numerous vessels found between outer epithelium and stratum intermedium without any penetration of latter. In 6 opposite wider portions of reticulum perivascular spaces and early penetration by vessels seen. Where reticulum is narrower in vicinity of cusps vessels penetrate more deeply toward stratum intermedium. (*Fig. 2*)
11	7 mos.	Tuberculous Meningitis	V, 6	In V crown is completely surrounded by "united epithelium" containing numerous blood vessels. Some approach within 10 micra of ameloblastic layer. Marked degenerative changes of ameloblasts which in some places seem to be associated with nearest approach of blood vessels evident. In 6 perivascular spaces are more marked than in Case 10. The vessels approach stratum intermedium. (*Fig. 5*)
12	11 mos.	Pneumonia, Acute Scurvy	6	Narrowing of reticulum with vascular penetration and appearance of perivascular spaces except on lateral aspects of crown. Papillae are found principally in areas with no vascular penetration of enamel organ and areas where large capsular blood vessels approach outer epithelium.
13	14 mos.	Acute Malnutrition	V	Stellate reticulum is missing except in small areas with large cystic cavities similar to and probably identical with perivascular spaces. A vessel found within the stratum intermedium less than 10 micra from ameloblasts. In 6 there is narrow reticulum with large perivascular spaces extending across its entire width. Blood vessels approach stratum but do not enter.

tion of the enamel organ may be seen in different teeth. The deciduous first molar may exhibit vascular invasion down to, and into, the stratum intermedium along with a complete disappearance of the reticulum except in the bottom of the occlusal grooves. In the same case the permanent first molar may show early penetration of the outer portion of a fully formed reticulum and many perivascular spaces arranged immediately beneath the papillary layer of the outer enamel epithelium.

One instance (patient 4) deserves particular mention because of the fact that vascular penetration was noted in the deciduous second incisor and canine in addition to the posterior teeth. This patient was 16 days old at the time of death from streptococcus septicemia. In the deciduous second incisor the stellate reticulum is greatly reduced in size over the cingulum and lingual fossa and is absent over the remainder of the crown. The papillary layer is composed of islands of epithelial cells which are practically separate from each other. In other areas, toward the incisal edge, the outer epithelium is so closely associated with the stratum intermedium that it cannot be identified as a separate layer. Over the lingual fossa where the reticulum is still 40 micra wide, many vessels are found midway between the stratum and the outer epithelium. In the deciduous canine blood vessels are seen entering the reticulum at a point near the border of the incisal third of the labial surface where the reticulum is 75 micra wide (*fig. 6*). No previous reports of vascular penetration of the enamel organ in the anterior teeth of man have appeared in the literature to our knowledge. Further study will be required to determine whether this is a normal occurrence.

DISCUSSION

Previous observers of the presence of blood vessels within the enamel organ of mammalian teeth may be classified into four categories: those who deny the vascular penetration at any period; those who maintain that vessels appear within the stratum intermedium but not within the reticulum; those who believe the penetration occurs only during involutionary changes while the reticulum is disappearing; and, lastly, that group, including the writer, who maintain that blood vessels enter the enamel organ before any involutionary

changes have begun and at approximately the time when amelogenesis is beginning.

There is a certain amount of agreement among those who have studied this question of the vascularity of the enamel organ with respect to the posterior teeth at a period when amelogenesis is going on. The fact that the blood vessels are first seen inside the enamel organ at approximately the time when the ameloblastic layer begins its activity strongly suggests, as Kingery states, "... that the vascularity of the enamel organ is closely associated with the processes of amelogenesis in these molar teeth and with the functions of the different parts of the enamel organ." Although no direct evidence has been brought forward to indicate just what the functions of the different components of the enamel organ are, the inferential evidence suggests the possibility that the outer epithelium serves as a sort of selective filter, absorbing and concentrating raw materials from the blood which will be utilized in amelogenesis.

The stellate reticulum appears to serve as a store for this material at the very beginning of enamel formation and as a means of transmitting this material to the deeper parts of the organ. The findings which support this hypothesis are as follows: (a) The development of a papillary layer in the outer enamel epithelium concurrently with a proliferation of the capsular capillary plexus around these papillae at a time when the ameloblastic layer is becoming active. (b) The degeneration of the reticulum concomitantly with the formation of enamel. (c) The invasion of blood vessels at approximately the time enamel formation begins or shortly thereafter, as if to replenish the material which had been stored within the stellate reticulum during the preformative period. (d) The apparent relation, as demonstrated by Kingery and others, between the degree of development of a papillary layer and the time of vascular penetration in different species—

"In the molars of the rat and the pig the early and extensive vascularization of the enamel organ renders unnecessary any high degree of development of the papillary layer. In the human molar, on the other hand, while the blood vessels enter the enamel organ early, there is no extensive vascularization until the enamel is well along in development. Consequently, the papillary layer is very highly developed and subserves the function of absorbing amelogenetic materials from the adjacent capillaries and transmitting these substances to the enamel organ until the plexus of blood vessels formed later within the

stellate reticulum brings the supply closer to the ameloblasts. The papillary layer, then, is apparently most highly developed in those teeth within the enamel organ of which there are no blood vessels or within which the blood vessels are found relatively late . . . ." (17).

(e) The apparent relation between vascularization and the relative areas of enamel-forming and material-absorbing layers offers further support to this hypothesis. Bolk's work on the marsupial molar indicates an early and extensive penetration of blood vessels. The molar of the marsupials has extremely elongated cusps with thick enamel and very deep grooves. In other words, in such a tooth enamel formation is taking place over a considerably greater area than that of the outer epithelium through which all of the amelogenetic material must pass. In the rat incisor, on the other hand, even though it erupts continuously, the papillary layer is considerably greater in area than the ameloblastic layer and is closely approximated to the ameloblastic layer, thus permitting the expeditious transfer of material from the former to the latter. No penetration occurs in the enamel organ of this tooth. In our human material, blood vessels penetrate the outer epithelium earliest on the inner surfaces of the cusps of forming enamel. This is interpreted as indicating that within the occlusal surface where the downward dipping enamel surface is larger than the overlying outer epithelium, the ameloblastic layer utilizes material faster than it can be absorbed and transmitted through the reticulum. Hence vascularization occurs earliest in this region. Vascular penetration on the lateral surfaces of the teeth occurs relatively late when the reticulum is reduced in width, because in this portion of the tooth germ, adequate amelogenetic material can be supplied by the outer epithelium over a somewhat longer period of enamel formation than upon the occlusal surface.

The present study can offer no further evidence than that previously reported upon the rôle of the blood vessels within the enamel organ during the process of involution of the stellate reticulum. Addison and Appleton, Kingery, and Santoné all state that a few mesenchymal cells enter the reticulum with the capillaries and assist in bringing about the loss of the reticulum. The presence of the large perivascular spaces may represent early foci of degeneration of the reticulum. These spaces may with even more probability represent perivascular lymphatic spaces comparable to the perivascular

spaces of the brain. They may conceivably play a part in the exchange of material between the blood and the stellate reticulum.

SUMMARY

1. Vascular penetration of the two intermediate layers in the enamel organs of the deciduous and permanent molars is almost invariably noted in infants up to fourteen months of age. Thirteen representative examples are reported. 2. The vascularization of the enamel organ occurs approximately at the time enamel formation begins or shortly thereafter. 3. The vessels appear first in the vicinity of the cusps, subsequently over the occlusal grooves and fossae, and only later, after a considerable amount of enamel has been formed, do they appear on the lateral surfaces of the teeth. 4. In one instance penetration of the stellate reticulum of a mandibular deciduous lateral and canine occurred before the outer enamel epithelium had come to lie upon the stratum intermedium. 5. The earliest examples of vascular penetration are associated with the presence of perivascular spaces. These may be either large lymphatic sinuses or areas of beginning resorption of the stellate reticulum. 6. In support of the suggestion of Lepkowski and Kingery a hypothesis is advanced that vascular penetration of the enamel organ in mammals depends first on the form of the tooth and its enamel-forming area with respect to that of the outer enamel epithelium and, second, on the amount of stellate reticulum and the extent of development of a papillary layer in the outer enamel epithelium.

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CONTENTS

I. Sustaining subscribers to the *Journal of Dental Research*, 1938. Compiled by the Publication Committee..... 519
II. International Association for Dental Research. Register of members by sections, as of Dec. 1, 1938. Compiled by the General Secretary..... 520

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14. *Louisville Section.*—Brooks Juett, H. T. Knighton, **R. E. Myers*, J. T. O'Rourke, W. M. Randall.—5.

15. *Minnesota Section.*—W. D. Armstrong, E. S. Best, P. J. Brekhus, J. T. Cohen, I. A. Epstein, B. S. Gardner, C. J. Grove, J. F. McClendon, F. S. Meyer, W. R. Schram, E. C. Stafne, N. O. Taylor, W. D. Vehe, H. F. Wahlquist, C. W. Waldron, **H. G. Worman*.—16.

16. *New Haven Section.*—**B. G. Anderson*, C. G. Burn, A. M. Crosby, J. P. Pigott, David Weisberger.—5.

17. *New Orleans Section.*—A. D. Brashear, S. L. Tiblier, **C. S. Tuller*, F. J. Wolfe.—4.

18. *New York Section.*—Edmund Applebaum, H. A. Bartels, Adolph Berger, F. E. Beube, Gerrit Bevelander, Theodor Blum, C. F. Bodecker,

W. A. Bossert, R. H. Brodsky, L. R. Cahn, M. W. Carr, G. W. Clapp, W. H. Crawford, C. G. Darlington, Gershom Downs, H. S. Dunning, W. B. Dunning, H. S. Dwyer, J. D. Eby, Genevieve Foley, Raymond Gettinger, W. J. Gies, W. K. Gregory, Milo Hellman, Samuel Greenberg, Samuel Hemley, I. Hirschfeld, Houghton Holliday, Franklin Hollander, R. G. Hutchinson, Jr., T. P. Hyatt, A. L. Johnson, Theodore Kaletsky, Maxwell Karshan, F. C. Kemple, Thomas B. King, Frances Krasnow, William Lefkowitz, H. J. Leonard, E. C. McBeath, J. O. McCall, F. S. McKay, A. H. Merritt, E. G. Miller, Egon Neustadt, **Isaac Neuwirth*, A. T. Newman, Edith Oblatt, B. B. Palmer, Theodor Rosebury, Irving Salman, J. A. Salzmann, M. I. Schamberg, Joseph Schroff, H. M. Shapiro, H. F. Silvers, Sidney Sorrin, F. L. Stanton, M. R. Stein, P. R. Stillman, David Tanchester, Benjamin Tenenbaum, L. M. Waugh, J. L. Zemsky, D. E. Ziskin.—65.

19. *Philadelphia Section.*—J. L. T. Appleton, Carrie K. Bryant, L. W. Burkett, H. R. Churchill, **T. J. Cook*, W. S. Crowell, L. I. Grossman, R. H. Ivy, J. V. Mershon, H. A. Miller, Hermann Prinz, C. R. Turner.—12.

20. *Pittsburg Section.*—W. H. Archer, T. W. Brand, G. J. Cox, J. J. Enright, W. L. Fickes, F. C. Friesell, H. E. Friesell, T. F. McBride, E. G. Meisel, J. S. Oartel, N. C. Ochsenhirt, W. F. Swanson, **L. E. VanKirk*, E. A. Wolf, W. H. Wright.—15.

21. *Prague Section.*—Karel Černý, Karl Häupl, Ján Jesenský, František Kostečka, Jaromir Krčcăn, Anton E. Loos, F. Náprstek, **Friedrich Neumann*, František Neuwirt, Čestimir Parma, Josef Přibyl, Ferdinand Škaloud, Karel Wachsmann, Jr., Karel Wachsmann, Hans Wermuth.—15.

22. *Richmond Section.*—Harry Bear, O. W. Clough, J. C. Forbes, J. F. Hall, A. P. Little, Harry Lyons, **Grant Van Huysen*, J. B. Williams.—8.

23. *Rochester Section.*—B. G. Bibby, R. E. Brawley, V. D. Cheyne, **M. L. Deakins*, H. C. Hodge, Paul Lalonde, Marian L. LeFevre, R. S. Manly, H. J. Sedwick, J. F. Volker, R. S. Voorhees.—11.

24. *St. Louis Section.*—L. R. Boling, E. P. Brady, O. W. Brandhorst, Edward Hampp, E. H. Keys, W. E. Koch, B. E. Lischer, L. R. Main, E. B. Owen, H. R. Raper, **H. B. G. Robinson*, R. C. Wheeler, J. D. White, G. B. Winter, W. B. Gurley.—15.

25. *San Francisco Section.*—G. L. Bean, Hermann Becks, W. C. Fleming, J. R. Gill, P. J. Hanzlik, G. M. Hollenbeck, G. A. Hughes, A. J. Ker, J. A. Marshall, G. S. Millberry, R. W. Rule, W. B. Ryder, Jr., F. W. Schubert, E. W. Schultz, Nina Simmonds, L. B. Taber, M. L. Tainter, W. W. Wainwright, R. G. Zeisz.—19.

26. *Toronto Section.*—H. K. Box, A. J. Broughton, Thomas Cowlings, **A. W. Ellis*, G. W. Grieve, F. C. Husband, A. D. Mason, A. J. McDonagh, E. W. Paul, H. S. Thomson.—10.

27. *Vienna Section.*—Arwed Berg, Karl Breitner, **Fritz Driak*, Bernhard Gottlieb, Richard Grohs, Otto Hofer, Ernst Kellner, Emmerich Kotanyi,

Hermann Mathis, Hans Pichler, Otto Preissecker, Franz Schönbauer, A. M. Schwarz, Harry Sicher, Karl Spring, Georg Stein, Richard Trauner, Hermann Wolf, Josef Weinmann.—19.

28. *Washington Section.*—F. A. Arnold, J. E. Ash, Ronald Barber, J. R. Beal, J. LeR. Bernier, C. W. Camalier, A. R. Crane, R. T. Dean, B. E. Erickson, L. P. Hartley, H. E. Harvey, Ales Hrdlicka, L. G. Jordan, Harry Kaplan, R. A. Keilty, **H. W. Krogh*, D. F. Lynch, S. V. Mead, G. C. Paffenbarger, I. C. Schoonover, Willmer Souder, E. R. Stone, W. T. Sweeney.—23.

29. *Winnipeg Section.*—**E. R. Bier*, G. A. Buchanan, F. T. Cadham, M. H. Garvin, K. M. Johnson, H. J. Merkeley, W. J. Riley.—7.

30. *Not now members of a section.—Amsterdam.*—T. E. de Jonge-Cohen, Elkan Sanders. *Boston.*—Benjamin Tishler. *Buffalo.*—Mathew Podolin. *Chicago.*—J. S. Shell. *Denver.*—K. K. Cross. *Fort Hancock.*—R. Wood Leigh. *Germany.*—Gustav Korkhaus, Freidrich Proell, Rudolph Weber. *Hartford.*—R. L. Coleman. *Korea.*—J. L. Boots. *Johannesburg.*—S. F. Gottlich. *Liverpool.*—G. G. Macphee. *Little Rock.*—S. N. Blackberg. *Milan.*—Gyula Csernyei, Silvio Palazzi. *Milford.*—Aaron Issaacs. *Montevideo.*—F. M. Pucci. *New York.*—H. W. Gillett. *New Zealand.*—C. D. M. Day, R. E. T. Hewat, R. M. S. Taylor. *Pittsburg.*—Faith P. Hadley. *Providence.*—A. L. Midgley. *San Francisco.*—E. H. Mauk, F. V. Simonton. *Saranac Lake.*—W. E. Taylor. *Swarthmore.*—J. A. Detlefson. *Tucson.*—Margaret C. Smith. *Washington.*—Henry Klein. *Zurich.*—Walter Hess.—32.

31. *Addresses as of Dec. 1, unknown.*—Martha R. Jones, Anna C. Nichols.—2.

Total number of members—421; average membership of twenty-nine sections—15.

Sectional meetings for 1938–39 have been scheduled as follows (data compiled by the General Secretary and incomplete to date): *Chicago*—Oct. 20, Jan. 19, May —. *Cleveland*—Dec. 21. *Iowa City*—Jan. —. *New York*—Jan. 19, June 1. *Philadelphia*—Oct. 29, Dec. 16. *St. Louis*—Dec. 15, Mar. 26. *Washington*—Nov. 29, March —, April 11.

The schedule for 1936–37 was as follows: Ann Arbor—May 12. *Baltimore*—Feb. 1. *Boston*—Jan. 18, Mar. 25. *Chengtu*—Mar. 25, May 27. *Chicago*—Nov. 20, Jan. 22, May 21. *Cleveland*—Feb. 26. *Columbus*—Mar. 5. *Iowa City*—Dec. 4, Mar. 8. *London*—Oct. 14, Feb. 3, Apr. 14. *Louisville*—Apr. 6. *Minnesota*—Dec. 15, Apr. 15, June 15. *New York*—Jan. 28, June 3. *Philadelphia*—May 14. *Pittsburgh*—Oct. 19, Jan. 11, Mar. 1, May 3. *Richmond*—Nov. 24, Feb. 17, Apr. 6. *Rochester*—Oct. 6, Jan. 6, Feb. 25, May 15. *St. Louis*—Dec. 1. *San Francisco*—July 13, Mar. 17. *Vienna*—Apr. 20. *Washington*—Nov. 30, Feb. 2, Apr. 13. *Winnipeg*—Oct. 12, Jan. 26, Mar. 8.

INDEX

Volume 17: 1938

In this index abstracts of papers presented at the Sixteenth General Meeting of the International Association for Dental Research are listed under the author and subject headings with the exception of abstracts for which no manuscripts were received which are listed only under the respective authors. Contributed articles are listed under the authors and subject headings.

SPECIAL ABBREVIATIONS: *A. C. D.*: American College of Dentists, *A. D. A.*: American Dental Association, *I. A. D. R.*: International Association for Dental Research, *J. D. R.*: Journal of Dental Research, *Minn.*: Minnesota.

A. C. D.: Gies dental fellowships, 317
——: greetings, 315
A. D. A.: caries; compilation of results, 314
ADAM, T. H.: caries; decalcification vs. injury, 95, 324
AISENBERG, M. S.: pulp; bone, 304, 371
ALLEN, F.: ptyalin; saccharification, 324
Amalgam: flow, 305, 332, 333
Ameloblastoma, 297, 328
Anacoresis: periapical infection, 319
ANDERSON, B. G.: arresting caries; clinical study, 443
Anesthetic: local; hemodynamic effect, 294
——: ——; mandibular injection, 125
——: ——; nerve effect, 300, 431
——: ——; sensitization, 295
——: ——; vasoconstrictor; toxicity, 295
Anomaly: accessory molar root, 319
Apicotomy: healing, 322
APPLEBAUM, E.: caries; decalcification vs. injury, 95, 324
——: enamel calcification; Grenz ray study, 181, 324
Arch: growth, 300, 349
——: form; Peruvian, 331
ARMSTRONG, W. D.: caries; fluorine, 301, 393
——: dentin and enamel; fluorine, 27, 301
——: muscles of mastication; stimulation, 292
Articulator: errors, 91
Ascorbic acid: parodontosis, 101, 308

BACTERIA carbohydrate degradation, 302
——: inhibitory agent; saliva, 308, 493
——: normal mouth, 57
——: oral; percentage occurrence, 265, 423, 471
——: pulp, 47, 311
See also under various organisms
BALE, W.: calcification; fluorescent phosphorus study, 312
BALKIN, S. G.: temperomandibular articulation; roentgenography, 296
BECKS, H.: saliva; activation, 324, 325
——: ——; phosphorus, 197, 217, 324
BENDER, I. B.: Gaucher's disease; dental relations, 359

BERRY, G. P.: saliva; effect on *L. acidophilus*, 493
BESSEY, O. A.: caries; guinea pig, 325
Beust, T. B.: necrology, 336
BEVELANDER, G.: calcification; parathyroidectomy, 310
BIBBY, B. G.: oral bacteria; different mouths, 423
——: ——; percentage occurrence, 265, 423, 471
——: saliva; antibacterial factor, 308, 493
Bite: strength, 292, 313
Black, A. D.: necrology, 337
BLAYNEY, J. R.: mouse tumor; dental, 297
BLEIKER, R. F.: temperomandibular articulation; ear disturbance, 325
Blood: ascorbic acid, 101, 308
——: clotting time; saliva method, 335
——: parodontosis, 15
——: pressure; local anesthetic, 294, 295
——: vessel; enamel organ, 311, 505
BLUM, T.: institute of oral pathology, 75
BODECKER, C. F.: enamel permeability, 309, 453
——: sodium perborate; effects of daily use, 161, 309
BOENHEIM, F.: parodontosis; endocrines, 19, 107
BOLING, L. R.: pulp; self-strangulation, 310
——: this index, 525
Bone: calcium, 31
——: Gaucher's disease, 359
——: pulp, 304, 371
——: tooth; age comparison, 330
BOUCHER, C. O.: articulators; errors, 91
BOYD, J. D.: dentin; specific gravity, 465
BOYLE, P. E.: caries; guinea pig, 325
——: parodontal tissues; guinea pig and man, 37
BRAWLEY, R. W.: saliva; calcium content; children, 293, 477
BREKHUS, P. J.: caries; fluorine, 301, 393
——: dentin and enamel; fluorine, 27, 301
——: muscles of mastication; stimulation, 292
BRENING, R. H.: halitosis; cryoscope and osmoscope, 314
BRODSKY, R. H.: Bucky-Grenz ray: parodontosis and tuberculosis, 326, 381
BROOKE, J. W.: bacteria; normal mouth, 57
BROWN, J. B.: saliva composition; secretion rate, 191
Bucky-Grenz ray, 326, 381
BUCKY, G.: Bucky Grenz ray; parodontosis and tuberculosis, 326
BUNTING, R. W.: I. A. D. R.; introduction of president, 290, 318
BURKET, L. W.: bacteria; oral distribution, 326
——: caries; diagnosis, 301

CALCIFICATION: enamel; Grenz ray study, 181, 324
——: enamel organ, 303
——: fluorescent phosphorus study, 312
——: parathyroidectomy, 310
Calcium: bone and tooth, 31
——: dentin; caries, 401
——: metabolism; oral manifestations, 295
——: saliva, 191, 293, 334, 477
Calculus: composition, 257
——: saliva, 334
CAHN, L. R.: sodium perborate; effects of daily use, 161, 309
CAMPAIGNE, E. E.: mucin plaque; synthesis, 302
Candy: enamel destruction, 499
Carbohydrate: degradation; bacteria, 302
——: ——; yeast, 302
Carbon dioxide: dentin; caries, 401
Caries: acidified candy, 499
——: arresting; clinical study, 443
——: compilation of results; A. D. A., 314
——: decalcification vs. injury, 95, 324
——: dentin composition, 401
——: diagnosis, 301
——: familial resemblance, 302
——: fluorine, 301, 393
——: guinea pig, 325
——: incidence, 327
——: plaque; pH, 251, 348
——: polarized light, 274
——: post-eruptive tooth age, 330
——: saliva factors, 299, 328
——: *S. viridans*, 296

Caries (*con.*):
——: tooth structure, 323
——: zones; composition, 303
See also bacteria, diet, nutrition, saliva, etc.
Casting: dimensional changes, 329
Cat: dental arch; growth, 349
Catalase: saliva, 294
Cement: mixture; deciduous teeth, 320
Cementum: coronal, 151, 304
——: infected teeth, 320
——: tooth fracture, 304, 371
CHASE, S. W.: growth rate; enamel prism stripes, 326
Children: caries; familial resemblance, 302
——: diet, 298
——: extraction incidence, 300
——: filling material, 320
——: saliva; calcium, 293, 477
CHEYNE, V. D.: extraction incidence; children, 300
——: ptyalin; saccharification study, 324
CLAFLIN, R. S.: tooth fracture; healing, 304
CLOUGH, O. W.: saliva; effect on *L. acidophilus*, 493
COHEN, J. T.: dental arches; growth, 300
COOK, T. J.: hemorrhagica purpura, 326
Cryoscope, 314
CSERNYEI, G.: pulpless teeth; therapy, 319
——: anacoresis; periapical infection, 319
CUMMINGS, E. C.: Chronological age; bone and tooth, 330
CUSHMAN, F. H.: diet; children, 298
Cyst: dentigerous, 328

DAMON, G.: Minn. St. D. A.; greetings, 316
DEAKINS, M. L.: dentin; specific gravity, 465
——: saliva; catalase, 294
Decalcification: caries, 95, 324
——: enamel, 499
Dentifrice: sodium perborate, 161, 309
Dentin: calcification; parathyroidectomy, 310
——: caries; composition, 401
——: fluorine, 27, 301, 393
Dentin (*con.*):
——: growth rate; rat, 307
——: hardness; variation, 297
——: molecular structure, 312
——: polarized light, 274
——: specific gravity, 465
DENTON, G. B.: extraction instrument; origin, 298
Diagnosis: caries, 301
DIEHL, H. S.: U. of Minn.; medical school; greetings, 317
Diet: caries, 313
——: children, 298
——: saliva, 411
Dog: radiography, 173
——: saliva, 293
DOWNS, W. G.: diet; effect on bones and teeth, 326
DRAIN, C. L.: dentin; specific gravity, 465
——: extraction incidence; children, 300
Drain, C. L.: necrology, 338
DRIAK, F.: filling material; deciduous teeth, 320
DUBÉ, V.: vulcanite; porosity, 327
DUNNING, J. M.: caries; incidence, 327

EAR disturbance: temperomandibular articulation, 325
EDWARDS, L. F.: tooth development; nerve resection, 115
Electric: current; oral, 305
——: pulp test, 79
Electrolysis, 321
Electroplating: impression material, 307
Enamel: calcification; Grenz rays, 181, 324
——: destruction; acidified candy, 499
——: fluorine, 27, 301, 393
——: molecular structure, 312
——: permeability, 309, 453
——: polarized light, 274
Enamel organ: microincineration, 303
——: vascularity, 311, 505
Endocrines: paradontosis, 19, 107
Epithelial attachment; rat, 323
ETHERINGTON, J. W.: caries; saliva secretion rate, 299
——: diet; children, 298
Extraction: incidence; children, 300

FACE: form; Peruvian, 331
Fellowship: Gies dental research, 317
FENSKE, E. L.: hardness; dentin; variation, 297
FIGI, F. A.: ameloblastoma; dentigerous cyst, 328
Fluorine: caries, 301, 393
——: dentin and enamel, 27, 301
——: effect of magnesium and sodium, 387
Fracture: tooth; healing, 304
FORD, G. S.: U. of Minn.; greetings, 316
FOSDICK, L. S.: carbohydrate degradation; bacteria, 302
——: ——; yeast, 302
——: mucin plaque; synthesis, 302
FRENCH, E. L.: caries; dentin composition, 401
Fusiform organisms, 53

GASSERIAN ganglion: injection, 322
Gaucher's disease: dental conditions, 359
Gies Dental Research Fellowships, 317
Gies Endowment Fund: report, 345
GIES, W. J.: caries; compilation of results; A. D. A., 314
GILLAM, E.: diet; growth; rat, 334
GLOCK, G. E.: salivary calculus; composition, 257
Gold: casting; dimensional changes, 329
GOLDSTEIN, H.: parodontosis; blood and systemic findings, 15
GORE, J. T.: saliva; enamel decalcification; autolysis, 411
——: saliva separator, 69
GOTTLIEB, B.: cementum; infected teeth, 320
GROHS, R.: catcher splint, 321
Growth: comparative; bones and teeth, 330
——: dental arches, 299, 349
——: facial; measurement, 299
——: fluoride effect, 387
——: rate, rat molar, 307
——: tooth; caries, 330
——: ——; nerve resection, 115
Guinea pig; caries, 325
——: parodontal tissues, 37

HABER, G.: diet; caries, 313
Halitosis, 314
HAMMOND, C.: caries; *S. viridans*, 296
HAMPP, E. G.: microincineration; enamel organ, 303
HANSEN, A. E.: calcium and phosphorus metabolism; disturbance; oral manifestations, 295
Hardness: dentin; variation, 297
HATTON, E. H.: I. A. D. R.; proceedings, 273
——: ——; list of members, 520
HILL, T. J.: I. A. D. R.; president; inaugural address, 291, 318
HODGE, H. C.: caries; dentin and composition, 401
——: molecular structure; dentin and enamel, 312
HOFER, O.: maxillary sinus; dental disease, 321
HOFFMAN, M. M.: growth rate; rat molar, 307
HOLLANDER, F.: caries; incidence, 327
HOLSTEIN, F. M.: dentin; internal resorption, 333
HOSKINS, M.: calcification; parathyroidectomy, 310
Hypoplasia: caries, 323

I. A. D. R.: executive proceedings, 339
——: greetings, 315
——: members, 520
——: ——; new, 343
——: necrology, 336
——: officers, 343
——: president; inaugural address, 291, 318
——: ——; introduction, 290, 318
——: ——; retiring; address, 274, 318
——: proceedings; abstracts, 292
——: ——; contents, 273
——: ——; index of participants, 292
Impression material: electroconduction, 307
Indian: Peru; face and arch form, 331
See also Eskimo
Inflammation: periapical; anacoresis, 319
——: pulp, 310, 332
See also bacteria, caries, etc.

Injury: caries, 95, 324
Iontophoresis, 321
Instrument: extraction; origin, 298
Investment: casting; permeability, 306

J. D. R.: board of editors; report, 342
——: committee on endowment; report, 341
——: publication committee; report, 342
——: ——; list of sustaining subscribers, 519
JONGE-COHEN, T. E.: molar; accessory root, 319
JUDY, F. R.: enamel destruction; acidified candy, 499
JUMP, E. B.: enamel organ; vascularity, 311, 505

KANNER, O.: bacteria; pulps of intact teeth, 47
——: galvanic current; oral, 305
KARSHAN, M.: saliva; calculus, 334
——: ——; caries, 328
KELLNER, E.: electrolysis and iontophoresis, 321
KITCHIN, P. C.: I. A. D. R.; retiring president's address; (polarization review), 274, 318
——: local anesthesia; effect on nerve, 300, 431
——: tooth development; nerve resection, 115
KLEIN, H.: caries; familial resemblance, 302
——: ——; post-eruptive tooth age, 330
KOCH, W. E., JR.: radiography; dogs, 173
——: this index, 525
KOMIVES, O.: mandible; variations, 125
KORNFELD, M.: castings; dimensional changes, 329
KOTANYI, E.: apicotomy; healing, 322
KRAMER, M.: caries; post-eruptive tooth age, 330
KRASNOW, F.: salivary protein, 329
KRONFELD, R.: coronal cementum and resorption, 151, 304

L. ACIDOPHILUS: saliva, 333
—: —; inhibition, 493
LASATER, R. L.: wax pattern; distortion, 306
LASBY, W. F.: U. of Minn.; dental school; greetings, 317
LEFEVRE, M.: calcification; fluorescent phosphorus study, 312
——: calcium; bone and tooth, 31
——: caries; dentin composition, 401
LEFKOWITZ, W.: enamel; permeability, 309, 453
LIMBACHER, H. P.: saliva composition; secretion rate, 191
LOSCH, P. K.: caries; saliva secretion rate, 299
——: chronological age; bones and teeth, 330
LUCKHARDT, A. B.: local anesthetic; hemodynamic effect, 294
——: ——; sensitization, 295

MAGNESIUM: effect on fluoride, 387
Mandible: variations, 125
MANLY, R. S.: caries' zones; composition, 303
MARGOLIS, H. I.: facial growth; measurement, 299
Mastication: caries, 443
Maxillary sinus: dental disease, 321
MCKEOWN, R.: diet; effect on bones and teeth, 326
Method: amalgam flow; measurement, 333
——: bacteria; oral; determination, 265, 333
——: blood clotting time, 335
——: breath examination, 314
——: calcium determination; bone and tooth, 31
——: chemical; caries' zones, 303
——: electric pulp testing, 79
——: growth; facial; measurement, 299
——: lactobacillus; estimation, 333
——: heat treatment; solder, 335
——: plaque; mucin; synthesis, 302
——: radiography; dogs, 173
——: saliva; separation, 69
——: ——; phosphorus, 235
——: specific gravity; dentin, 465

MIDGLEY, A. L.: Gies dental research fellowships and awards; A. C. D., 317
Minn.: State D. A.; greetings, 316
——: University; greetings, 316
——: ——; dental school, greetings, 317
——: ——; medical school, greetings, 317
MORSE, F. W., JR.: ascorbic acid; parodontosis, 101
——: bacteria; pulp, 311
——: chronological age; bones and teeth, 330
Mottled enamel: fluorine, 27
See also fluorine
Mouse: tumor; dental, 297
Mucous membrane: effects of sodium perborate, 161, 309
MURRAY, M. M.: salivary calculus; composition, 257
Muscles: mastication; stimulation, 292
MYERS, R. E.: casting investments; permeability, 306

NERVE: effect of local anesthetic, 300, 431
——: resection; tooth development, 115
NICHOLSON, E.: calcium; bone and tooth, 31

OBLATT, E. B.: salivary protein, 329
Osmoscope, 314
Osmotic pressure: local anesthetic; nerve effect, 300, 431

PALMER, C. E.: caries; familial resemblance, 302
——: ——; post-eruptive tooth age, 330
Parathyroidectomy: calcification, 310
Paradontal tissues: guinea pig and man, 37
Paradontosis: ascorbic acid, 101, 308
——: blood and systemic findings, 15
——: Bucky-Grenz ray, 381
——: endocrines, 19, 107
Pathology: oral; institute, 75
Peridental membrane: bone formation, 304, 371
——: fracture reaction, 304
See also parodontal tissues
PFEIFFER, K. R.: casting investments; permeability, 306
pH: dental plaque, 251, 348
——: local anesthetic; nerve effect, 300, 431
——: saliva, 293, 334
Phosphorus: dentin; caries, 401
——: fluorescent; calcification, 312
——: metabolism; oral manifestations, 295
——: saliva, 191, 197, 217, 235, 334
PICHLER, H.: Gasserian ganglion; injection, 322
PICKERING, P. P.: local anesthetic; hemodynamic effect, 294
——: ——; sensitization, 295
Plaque: dental; pH, 251, 348
——: mucin; synthesis, 302
Polarized light: tooth tissues, 274
PRICE, W. A.: arch and face form; Peruvian; ancient, 331
——: ——; ——; comparative study, 331
Protein: saliva, 329, 334
Pulp: bacteria, 47, 311
——: bone, 304, 371
——: canal therapy, 319
——: reaction, 79
——: pathology, 332
——: self-strangulation, 310
——: testing; electric, 79

RADIOGRAPHY: age comparison; bones and teeth, 330
——: Bucky-Grenz ray; parodontosis and tuberculosis, 381
——: caries' diagnosis, 301
——: dogs, 173
——: Gaucher's disease, 359
——: Grenz; enamel calcification, 181, 324
——: laminograph, 325
——: tongue position, 313
Rat: caries; decalcification vs. injury, 95, 324
——: epithelial attachment, 323
——: molar; growth rate, 307
Resorption: coronal, 151, 304
Rhinoceros: fossil; shortening tooth series, 1
RITTERSHOFER, L. S.: pulp pathology, 332

ROBINSON, H. B. G.: pulp; self-strangulation, 310
——: this index, 525
Roentgenography. *See* radiography
Root: accessory; molar, 319
Root canal. *See* pulp
RUDOLPH, C. E.: A. C. D.; greetings, 315

SALIVA: antibacterial agent, 308, 493
——: autolysis, 411
——: blood clotting time, 335
——: buffer action, 411
——: calcium, 293, 334, 477
——: calculus, 334
——: carbohydrate content, 411
——: caries factors, 328
——: catalase, 294
——: comparative study, 293
——: diastatic action, 411
——: lactobacillus, 333
——: mandibular, 69
——: parotid, 69
——: pH, 334
——: phosphorus, 191, 197, 217, 235, 334
——: protein, 329, 334
——: secretion rate; caries, 299
——: ——: composition, 191, 294
——: separator, 69
SCHÖNBAUER, F.: gingival pockets; therapy, 323
SCHOUR, I.: growth rate; rat molar, 307
SCHUCK, C.: fluoride; effect of magnesium and sodium, 387
SEDWICK, H. J.: saliva; calcium content; children, 293, 477
SHAPIRO, H. H.: dental arch; growth; cat, 349
SIMMONS, E. J.: caries; dentin composition, 401
SIMON, B.: mandible; variations, 125
SIMON, W. J.: muscles of mastication; stimulation, 292
SKINNER, E. W.: amalgam flow, 305, 332, 333
——: soldered joints; treatment, 335
——: vulcanite; porosity, 327
SMITH, M. C.: diet; growth; rat, 334
SNYDER, M. L.: lactobacillus; saliva estimation, 333
Sodium: effect on fluoride, 387
Sodium perborate: effects of daily use, 161, 309
Solder: heat treatment, 335
Specific gravity: dentin, 465
STAFNE, E. C.: ameloblastoma; dentigerous cyst, 328
STEIN, G.: caries; tooth structure, 323
STEINMEYER, H. P.: local anesthetic; hemodynamic effect, 294
STEPHAN, R. M.: dental plaque; pH, 251, 348
Streptococcus viridans; caries, 296
SULSER, G. F.: halitosis; cryoscope; osmoscope, 314
SWANSON, W. F.: dentin; internal resorption, 333
Systemic diseases: parodontosis, 15, 19, 101, 107, 308
Szabo, J.: necrology, 339

TAINTER, M. L.: local anesthetic; vasoconstrictor; toxicity, 295
TAYLOR, P. B.: vulcanite; porosity, 334
Temperomandibular articulation: ear disturbance, 325
TENNENBAUM, B.: saliva; calculus, 334
THAYER, P. L.: bacteria; pulp, 311
Therapy: Bucky-Grenz ray, 381
——: caries; conservative, 443
——: Gasserian ganglion injection, 322
——: paradontosis; endocrine, 107
——: ——; vitamin C, 101, 308
——: pulp canal; electric, 321
——: pulpless teeth, 319
THOMA, K. H.: osteogenic tumors, 334
THOMPSON, G. E.: impression material; electroconduction, 307
THOMPSON, J. R.: tongue; position, 313
THRONDSON, A. H.: local anesthetics; vasoconstrictors; toxicity, 295
Tongue: position, 313
Tooth: bone age; comparison, 330
——: calcium, 31
——: development; nerve resection, 115
——: movement; age, 1

Toxicity: local anesthetic; vasoconstrictor, 295
TRIMBLE, H. C.: caries; saliva secretion rate, 299
Tuberculosis: oral; Bucky-Grenz ray, 381
Tumor: ameloblastoma, 297, 328
——: dental; mouse, 297
TUNNICLIFF, R.: caries; *S. viridans*, 296
——: fusiform organisms, 53
TURBYFILL, W. J.: soldered joints; treatment, 335

VAN KIRK, L. E.: dentin; internal resorption, 333
Vasoconstrictor: local anesthetic; toxicity, 295
Vincent's infection: bacteria, 57
See also fusiform organisms
VISSCHER, M. B.: saliva; comparative study, 293
Vitality: enamel, 309, 453
Vitamin C: parodontosis, 101, 308
Vitamins: caries, 325
VOLKER, J. F.: blood clotting time; method; saliva, 335
Vulcanite: porosity, 327

WAINWRIGHT, W. W.: saliva; phosphorus, 197, 235, 324
——: salivary analysis; biometric, 335
WALD, A.: pulp testing; electric, 79
WALDRON, C. W.: giant cell tumors, 300
WAUGH, L. M.: Eskimo; bite, 310
——: ——; caries and diet, 335
——: William J. Gies Endowment Fund; report, 345
Wax pattern: distortion, 306
WEAVER, H. M.: local anesthetics; effect on nerve, 300, 431
WEINMANN, J.: epithelial attachment; rat, 323
WEISBERGER, D.: ascorbic acid; parodontosis, 101, 308
WELCH, E. A.: caries; dentin composition, 401
WESSINGER, G. D.: carbohydrate degradation; yeast, 302
WEST, E. S.: enamel destruction; acidified candy, 499
WOOD, H. E., 2ND: shortening tooth series with age; causal factors, 1
WORMAN, H. J.: pulp pathology; etiology, 336
WRIGHT, H. N.: hardness; dentin; variation, 297
WRIGHT, H. D.: saliva composition; secretion rate, 191

X-RAY. See radiography

YATES, M. F.: bacteria; pulp, 311
Yeast: carbohydrate degradation, 302
YOUNG, A. P.: ascorbic acid; parodontosis, 101

ZEMSKY, J. L.: osteosclerosis; oral, 336
ZISKIN, D. E.: pulp testing; electric, 79

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